XT
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vol 61, no.1

Transactions

of the

Illinois State Academy of Science

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Volume 61
No. 1
1968

Springfield, Illinois

TRANSACTIONS of the ILLINOIS STATE ACADEMY OF SCIENCE

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(46509—11-68)

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TRANSACTIONS

OF THE

ILLINOIS STATE ACADEMY OF SCIENCE

VOLUME 61 - 1968 No. 1

ILLINOIS STATE ACADEMY OF SCIENCE

AFFILIATED WITH THE

ILLINOIS STATE MUSEUM DIVISION

SPRINGFIELD, ILLINOIS

PRINTED BY AUTHORITY OF THE STATE OF ILLINOIS

OTTO KERNER, *Governor*

March 1, 1968

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ISOLATION OF SECRETIN AND CHOLECYSTOKININ CONCENTRATES

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ABSTRACT. — Concentrates containing secretin and cholecystokinin have been prepared from a salted acid extract of the upper intestine (A-ppt) of the pig. The latter was extracted with 80% ethanol, the alcohol removed under vacuum and the residue submitted to isoelectric precipitation at pH 5.4 and heating and the activity precipitated from the resulting cooled filtrate (Filt I) by trichloroacetic acid. Filt I was also treated with various adsorbents or chloroform prior to the addition of trichloroacetic acid. Secretin and cholecystokinin in the resulting solids could be further enriched by the addition of amines in aqueous acetone solution. The studies were also extended to other animal species, including those without gall bladder.

Secretin, the hormone stimulating pancreatic secretion and choleresis and cholecystokinin (CCK), the principle eliciting contraction of smooth muscle, notably that of the gall bladder, occur simultaneously in a variety of intestinal extracts. By certain techniques, a preferential enrichment can be afforded. Thus, with the A-precipitate (A-ppt.), a salted acid extract of the upper intestine (Weaver *et al.*, 1926), extraction with media of mounting alcohol concentration progressively decreased the CCK unitage removed (Lueth *et al.*, 1938; Gershbein and Krup, 1952). The reader is referred to several reviews for the earlier literature (Greengard, 1948; Grossman, 1950; Grossman, 1958). According to Ågren (1939), a separation of secretin and CCK was claimed with precipitating agents on the basis of a purported difference in their isoelectric points, being 7.75 and 5.0-5.5 for secretin and CCK, respectively. Gershbein and Krup (1952) showed that the acetone-dried A-ppt (DA) comprises an effective starting material for the isolation of secretin concentrates low in CCK, especially by use of higher concentrations of ethyl alcohol and in conjunction with several other procedures. The hormonal activity of the A-ppt has been precipitated by means of trichloroacetic acid (TCA), alcohol extraction and isoelectric precipitation being employed in the preliminary processing (Greengard and Ivy, 1938). Such products (SI) were further enriched by steps culminating in a "crystalline" secretin picrolonate, the identity of which was later disproved (Greengard *et al.*, 1947). Aside from SI, the occurrence and relative distribution of CCK in the respective fractions derived from this concentrate were not explored by the latter workers.

Fishman (1959) found column chromatography on Dowex 50 and

IRC-50 or paper and starch electrophoresis to be unsuitable in secretin purification starting with a commercial (Lilly) product. Better success was achieved with electrophoresis at pH 7.5, polyvinyl powder serving as the support. By paper electrophoresis, the isoelectric point occurred in the range of 10-11 and a minimal molecular weight of 4400 was deduced on the basis of amino acid analysis. Such determinations have also been carried out by Jorpes and Mutt on their potent secretin products and procedures have been advanced by them for the isolation of the latter as well as pancreozymin-CCK fractions (Jorpes and Mutt, 1955, 1961, 1966; Jorpes *et al.*, 1964; Mutt, 1959).

The present study was undertaken with the view of determining the relative distribution or ratio of secretin to CCK in several concentrates originating from hog A-ppt and to extend such observations to other species. In addition, the purpose was to obtain concentrates containing both secretin and CCK by rather simple techniques toward use in special metabolic and pharmacological studies rather than in the isolation of very active small or simpler molecular types. The assay of secretin and CCK was greatly enhanced by use of the method of isobollic dosages (Gershbein *et al.*, 1949, 1953).

Materials and Methods

The first 3-5 ft of hog small intestine was removed soon after slaughter. The everted strips were freed of any contents and allowed to remain in contact with cold 0.4% hydrochloric acid (200 ml/strip) for 20 min with frequent stirring. They were wrung out by hand and the fluid filtered through cheese cloth and saturated with sodium chloride. Filtration yielded the A-ppt. It was not dried with acetone but used as such. The adsorbents comprised Lloyds' Reagent (Lilly), Permutit (according to Folin), Permutit S, Deacidite 735 and IR-100 (Resinous Products and Chemical Company). The aluminum hydroxide suspension, prepared by the addition of 1% ammonium hydroxide to 1% alum solution according to the Tracy and Welker procedure (1915), yielded 0.75-0.90 g aluminum oxide per 100 g gel on ignition. In general, filtration of concentrates and washing with solvents was achieved by centrifugation. Final drying was carried out over sodium hydroxide pellets under vacuum. The products were stored in the cold.

SI Concentrates

Alcohol extraction of the A-ppt.-The A-ppt was blended in a Waring blendor with 80% ethanol (10 ml/g) and the contents filtered and the residue again extracted with ethanol in the above amount (Greengard and Ivy, 1938). The supernatant fluid was rid of alcohol under reduced pressure at a temperature under 30°C. With the larger batches, the alcoholic suspension was filtered in a Sharples centrifuge and the filtrate freed of alcohol in a glass-lined vacuum still of 100 or 200 1. capacity. The resulting aqueous mixture with a pH generally around 1.5-2.0 was treated with aqueous sodium hydroxide to a pH of 5.5, rapidly heated to boiling with live steam, filtered and the resulting filtrate immediately chilled (Filt. I). The activity was precipitated by the addition of 6N trichloroacetic acid to a final concentration of 5% or after preliminary processing with chloroform or adsorbents. Except for some of the factor studies, the contents were filtered after chilling over a period up to 3 days. The precipitate (SI) was collected by centrifugation, washed with 1.7:1 acetone-ether by volume, 1.7:1 ether-acetone, and finally ether. In several runs, one volume of Filt. I was added to 10-15 volumes acetone and the resulting product after chilling for 24 hr, was filtered by centrifugation and washed with 1:1 ether-acetone portions and finally ether.

Pretreatment with Chloroform.-Filt. I was shaken with 10-20% of its volume of chloroform and the resulting milky suspension broken by a short period of centrifugation; solid occurred at the interface. The top layer was removed and again shaken with chloroform, then

processed with trichloroacetic acid as in the above.

Pretreatment with Adsorbents.—Portions of Filt. I were passed over the respective adsorbent columns and the resulting filtrates were then treated with TCA. Equal volumes of aluminum hydroxide suspension and Filt. I were shaken frequently over a period up to 1 hr before centrifugation; TCA was added to the resulting filtrate. The yellow to amber color of Filt. I was greatly reduced in intensity upon addition of some of the adsorbents, especially the aluminum hydroxide mixture (Al-Susp).

Treatment of SI with Amines and Picric Acid

The procedure of Greengard and Ivy (1938) was employed with modification in the treatment of SI with aniline in aqueous acetone media. The contents were filtered and the aniline precipitate (AP) extracted two more times with the amine mixture. The combined filtrate was freed of aniline and acetone under reduced pressure, several additions of water being required for removal of the free amine. On introduction of the aqueous filtrate into excess acetone, a solid (VII) separated. In a few cases, the aqueous filtrate was filtered after 16 hr of chilling. The aniline precipitate, a source of the hormone pancreozymin, was washed with acetone, 1:1 acetone-ether and then ether. With several SI concentrates, dimethylamine and triethylamine were substituted for aniline. Removal of excesses of these amines was relatively simple because of the lower boiling points. Aqueous solutions of SI on treatment with picric acid in acetone led to the separation of precipitates which could be fractionated by 70% acidified acetone.

Assay Procedures

The method of Gershbein *et al.*, (1949) was employed in the assay of secretin and CCK, 2-3 dogs being used per sample. The pancreatic duct of the pentabarbitalized animal was cannulated and a trocar secured in the dome of the gall bladder after clamping the cystic duct. A sample of hog SI adopted as the standard was arbitrarily assigned a unitage of 1.0 for both secretin and CCK. For secretin, the ratio of submaximal dosages of standard to unknown, each eliciting the same drop rate over a period of 10 min. was equated to the unitage of the test extract. Similarly, the ratio of dosages of either product yielding the same submaximal gall bladder contraction was taken as the CCK unitage of the unknown. For the latter hormone, the *in vitro* assay procedure based on the isolated guinea pig gall bladder was also applied for check purposes (Gershbein *et al.*, 1953); the log dose vs. response curve was widely employed and where the condition of the organ changed, a new curve was derived.

Results

Series A. SI-36.—The processing of 2 kg hog A-ppt by the usual procedure described above yielded 27.73 g of water-soluble powder, SI-36, assaying 4.0 u/mg each of secretin and CCK.

Fractionation with Aniline. — To each of two 500 ml centrifuge bottles containing 4.00 g SI-36 was added with stirring 80 ml water for solution then 320 ml acetone, 0.08 ml concentrated HCl and finally 10 ml freshly distilled aniline. The contents were centrifuged and the supernatant fluid removed. The precipitate was treated with aniline in aqueous acetone two more times and the filtrates combined and concentrated under vacuum; the dried aniline precipitate weighed 3.33 g.

The residual fluid (400 ml) after removal of the free amine was extracted with 280 ml n-butyl alcohol and the emulsion broken by centrifugation at 1500 rpm for 5 min; a negligible amount of interfacial solid resulted. The upper or butyl alcohol layer (BuOH-Ext-I) was set aside and the water layer again extracted with 280 ml of the alcohol to yield the second portion, BuOH-Ext-II. The aqueous solution was rid of alcohol under vacuum and the resulting clear fluid with washings (425 ml) introduced into 5.9 l acetone. A light solid separated after 5 min in

the cold and was filtered two days later; yield of white water-soluble solid, VII-36A: 1400 mg (assay: 12.5 u/mg each of secretin and CCK). BuOH-Ext-I was concentrated under vacuum and the orange-red residue taken up in portions of absolute methyl alcohol totalling 12 ml. On addition of the filtrate to 200 ml anhydrous ether, a solid resulted (BuOH-I-Sol; 188 mg) which assayed for 18 u/mg each of secretin and CCK; the insoluble material (BuOH-I-Insol) weighed 39.4 mg. Under the same conditions, BuOH-Ext II yielded 5.2 mg of essentially inert methyl alcohol-insoluble solid (BuOH-II-Ins.) and 57.6 mg of BuOH-II-Sol (assay: secretin, 11 u/mg.; CCK, $<<11$ u/mg).

Series B. SI Concentrates.—Filt. I in amount of 11.87 l obtained from the processing of 4.0 kg A-ppt. was submitted portionwise to various treatments before the addition of TCA as shown in Table I. An aliquot (3.56 l) was divided among 12 centrifuge bottles and each shaken with 30 ml chloroform. The aqueous layers were separated from the interfacial solid after centrifugation (PR-50; 2.30 g). A second treatment with chloroform produced essentially no solid. The addition of TCA to the filtrate yielded 6.26 g of SI-50.

In the treatment of 400 ml Filt. I with 400 ml Al-Susp, the mixture which was shaken over a period of 1 hr was centrifuged. The clear filtrate (710 ml) on treatment with TCA gave rise to 188 mg of SI-54 (assay for secretin and CCK in u/mg: 9.7 and 9.5, respectively).

Portions of Filt. I in amount of 400 ml were treated batchwise and with stirring over a period of 2 hr with 12 g Lloyds' Reagent, 23 g Zeo-Karb H and 23 g IR-100 and after filtration, TCA was introduced into the respective mixtures and the SI concentrates processed. The yields and assay data are presented in Table 1.

Treatment of SI-47 with Amines. —*Aniline.* Enrichment of hormonal activity of SI-47 was carried out with aniline as per the procedure for Series A except that n-butyl alcohol extraction was deleted. The final filtrate and washings from 2.00 g SI-47 after removal of the excess amine amounted to 72 ml and on introduction into 1.4 l acetone yielded 380 mg VII-46A (assay for secretin and CCK: 9.1 u/mg each).

Triethylamine. Similar to the last experiment, 1.00 g SI-47 was deproteinized in aqueous acetone solution with 5 ml redistilled triethylamine. The final filtrate (36 ml) on treatment with 700 ml acetone gave rise to 249 mg solid (VII-46B; CCK and secretin: 7.6 u/mg).

Dimethylamine. The last run was repeated in all details except that 3.5 ml anhydrous dimethylamine was used in each extraction. The yield of VII-46C was 281 mg and contained either hormonal activity at 7.4 u/mg.

Treatment of SI-50 with Aniline as such and in Conjunction with Al-Susp.-A total of 4.00 SI-50 (secretin: 2.5 u/mg; CCK: 4.0 u/mg) was treated with aniline in aqueous acetone and the final filtrate and washings after concentration under vacuum amounted to 150 ml. Of the latter, 75 ml was introduced into 1.4 l acetone. Yield of VII-50-N: 329 mg. (secretin: 3.0 u/mg; CCK: 11 u/mg). The remaining 75 ml was shaken with Al-Susp (75 ml) over a

period of 35 min and the resulting filtrate concentrated to a volume of 75 ml under vacuum. Treatment with acetone led to the separation of 248 mg of VII-50-CR (assay: secretin, 2.3 u/mg; CCK, 5.9 u/mg).

Series C and D. SA and SI Concentrates.—The use of chloroform and Al-Susp in conjunction with Filt I was further explored in Series C and D (Table 1), employing two additional batches of porcine A-ppt. A second addition of TCA to the filtrate after removal of SI-RIOA precipitated SI-RIOB. The SA products were obtained by adding an aliquot of Filt I to a 10-15 fold excess of acetone and chilling the contents for 24 hr prior to filtration.

The interfacial solid resulting on shaking 100 ml of Filt. I of Series C with 20 ml chloroform and centrifuging amounted to 42.9 mg. The filtrate after removal of SI-R9 when treated with a comparable volume of TCA, yielded 6.9 mg of product (SI-R9B).

SI Concentrates of Series D Employing Combined Chloroform and Al-Susp Treatments.—*Experiments 84T and 85T.* A total of 810 ml Filt. I was shaken with 810 ml Al-Susp over 1 hr after which time the contents were filtered; volume of solution A: 1245 ml. Of the latter, 945 ml was submitted to treatment with TCA and SI-84 removed (208 mg; assay: secretin and CCK, 16.0 u/mg each). The remainder of solution A (300 ml) was shaken with 25 ml chloroform and centrifuged; a minor amount of the interfacial solid was present. The aqueous filtrate on processing yielded 87.0 mg of SI-85 (assay: secretin and CCK: 12.6 u/mg each).

Experiments 86T and 88T. Filtrate I (400 ml) was shaken with 25 ml chloroform and the aqueous layer separated from the interfacial solid (PR-88; 424 mg). Of the filtrate (395 ml: solution B), 95 ml was treated with TCA, yielding 102 mg of SI-88 (assay: secretin, 4.0 u/mg; CCK, 6.8 u/mg). The remaining solution B was shaken with Al-Susp (300 ml) and the final filtrate in amount of 522 gave rise to 30.8 mg SI-90 (assay: secretin, 6.3 u/mg; CCK, 9.6 u/mg).

Further extraction of the A-ppt of Series D.—The A-ppt after the usual two extractions with 80% ethanol was submitted to a third one with 3 1. Processing yielded 5.88 mg SI-77 containing secretin and CCK at 4.0 u/mg each.

Series E. The SA and usual SI concentrates were isolated from Filt. I of yet another collection of porcine A-ppt and the activity compared with the SI products precipitated from the 100 ml aliquots of filtrate passed over columns containing Deacidite 735, Permutit S and Permutit according to Folin. Under these conditions, the latter yielded no precipitate on TCA addition.

Fractionation of SI Concentrates with Picric Acid. Experiment 91P.—SI-36 in amount of 501 mg was dissolved in 20 ml water and treated with a warm mixture of 3.5 g picric acid in 2.5 ml acetone. The resulting precipitate was removed after 16 hr and blended with 20 ml of 70% acidified acetone (composition: 20 ml concentrated HCl, 280 ml water and 700 ml acetone). The filtrate was separated from the solid (P-36-I), introduced into 275 ml acetone and the precipitate washed with acetone un-

til free of picric acid (P-36-S; 286 mg; assay: secretin, 3.3 u/mg and CCK, 0.55 u/mg). The insoluble material, P-36-I, on processing, weighed 123 mg and contained secretin at 1.1 u/mg and CCK at 1.4 u/mg. The overall recoveries of secretin and CCK based on the starting SI were 53 and 16%, respectively.

Experiment 95P.—Similar to the above, SI-47 (499 mg; 1796 u) yielded the soluble product, P-47-S (227 mg; secretin, 2.9 u/mg and CCK, 0.62 u/mg) and the insoluble portion (105 mg; secretin, 1.1 u/mg and CCK, 0.91 u/mg) or a recovery of 774 u (43%) secretin and 237 u (13%) CCK.

Experimment 97P.—The soluble product, P-79-S, arising from SI-79 (386 mg; 2162 u), weighed 215 mg (assay: secretin, 4.0 u/mg; CCK, 0.62 u/mg or recoveries of 40 and 6%, respectively).

Hormonal Concentrates from Other Animal Species. The isolation of SI and allied concentrates from the A-ppt was extended to several other species of either sex. The A-ppt which averaged up to 15 g per upper intestinal strip (3-5 ft lengths) for the hog ranged higher with comparable lengths from cattle and horses. The ratio of secretin to CCK was also close to unity employing the porcine SI standard. From thirteen 15 inch intestinal lengths from rabbits, the yield of SI was 95 mg and assayed 0.57 u/mg each for secretin and CCK. With two collections of beef intestines (22 and 17 strips, respectively), the yields of SI were 413 mg (secretin and CCK: 2.7 u/mg each) and 221 mg (either hormonal activity: 5.3 u/mg), in the order stated. Comparable activities were obtained with canine and ovine SI. Thus, with 30-2 ft intestinal strips from sheep of either sex, the SI concentrate (118 mg; losses) assayed 4.4 u/mg each for secretin and CCK. The yield of SI from 2 macaques (10 inch strips) was 41.0 mg containing each principle at 2.0 u/mg. Data for concentrates obtained from rats, elk and horses, animals without gall bladder, appear in Table 2. All collections were made within 20 min after killing except for a period of 6 hr elapsing between killing of the elk cows in the field and acid extraction of the upper 6 ft of intestine. The everted rat upper intestinal strips were about 2 inches in length. The respective secretin: CCK ratios underwent little change when representative VII-concentrates were prepared.

Discussion

The treatment of the upper intestinal extract, the A-ppt, with two aqueous ethanol portions, removal of solvent under vacuum and isoelectric precipitation at pH 5.5 and steaming of the contents leads to Filt I. In the present study, the latter has been submitted to several fractionation schemes preliminary to precipitation of the activity with TCA to yield the SI products. The crudest concentrates (SA) obtained by addition of Filt I to excess acetone are high in salt and the ratio of secretin to CCK is virtually unity. As the over-all unitage of secretin and CCK in SA is quite close to the values for SI by the conventional method, such solids may be employed in gauging and checking expected activities. Although these concentrates have good

storage qualities, several proved toxic to the assay dog.

In the treatment of Filt. I with TCA to a final concentration of 5%, the SI precipitate forms very rapidly. When the TCA content was increased to 10%, the activities of secretin and CCK did not differ from the conventional SI but a final level of 25% caused destruction of about 30% of either hormonal activity (SI-45; Table 1).

When Filt. I was shaken with chloroform and the suspension broken by centrifugation, removal of the aqueous portion from the interfacial solid and treatment with TCA, yielded SI products in which CCK was definitely higher than the secretin level. The ratios of CCK:secretin amounted to 1.6-2.0. The interfacial solid which displayed an elevation in secretin activity was quite toxic to several of the assay animals. The greatest enrichment of Filt. I was achieved by means of Al-Susp. The resulting fluid after filtration led to SI concentrates with at least three-fold increases in both secretin and CCK activity but with concomitant diminution on a weight or over-all unitage basis. It will also be noted that the 1:1 ratio of CCK:secretin of SI was not altered by the Al-Susp procedure or by deproteinization with aniline, nor on subsequent shaking of the Al-Susp-treated filtrate with chloroform prior to TCA addition. A significant decrease in either hormonal activity was suffered by Filt. I (Series D; Table 1) when the supernatant liquid from the Al-Susp step was submitted to treatment with chloroform or when the order of the two treatments was reversed as summarized in Table 3. In this connection, the addition of Al-Susp to the aqueous residue from SI-50 following removal of aniline, led to a poor yield of concentrate (VII-50-CR) with concomitant diminution in both secretin and CCK as compared to the usual product or VII-50-N; the ratio of CCK to secretin fell from 3.7 to 2.6. These findings lend further support to the contention of the writer that the nature, type and relative distribution of matrix impurities greatly affect the efficacy of various combinations of fractionation procedures. The reader is referred to an earlier article for further illustrations and discussion (Gershbein and Krup, 1952).

The SI-aniline deproteinization procedure of Greengard and Ivy (1938) as modified in this study, gives rise to concentrates (VII) with effective enrichment. They are amenable to clinical application, vasodilatins and pyrogen being essentially absent, at least, at the 'higher' human dosage levels. No pharmacological advantage is afforded by extraction of the aqueous filtrate after aniline distillation with n-butyl alcohol and in fact, such steps contribute toward lower yields. The aniline precipitate by-product contains low levels of secretin and CCK (ratio: about 1) but comprises a good source of pancreozymin, the principle which elicits a pancreatic secretion high in enzyme content. Reactions in several dogs have been encountered in the past on injection of moderate dosages of several of these concentrates. The yields of VII are not unequivocally predictable. The substitution of lower water-soluble amines toward deproteinization of SI has been instituted to circumvent

the rather noxious aniline. Such amines as triethylamine and dimethylamine yield VII-concentrates of somewhat lower but comparable potency and accordingly, show promise in fractionation schemes.

The solid exchange resins and adsorbents used in this study caused little enrichment of hormonal concentrates either by batch or column techniques except for Lloyds' Reagent and Permutit according to Folin. Under the present conditions, Lloyds' Reagent adsorbed virtually all of the activity but this material merits further investigation since the unadsorbed 'inactive' portion constituted about 50% of the conventional SI on a weight basis (Table 1). With Permutit according to Folin, no solid could be recovered after TCA treatment.

Although the recovery of hormonal activity was low, the treatment of the conventional SI in aqueous solution with picric acid and fractionation of the solid with acidified acetone, led to a secretin: CCK ratio which for the portion soluble in this medium was no longer unity, but ranged 4.7-6.6. For the resulting insoluble fraction, the ratio was about 1.

The findings with SI concentrates isolated from various species are of interest. Although the occurrence of CCK has been demonstrated in a number of animals, the relative distribution of the two principles was currently investigated. On the basis of the porcine SI standard, prepared by the usual method and assigned a value of 1.0 u/mg each of secretin and CCK, good correspondence or a similar ratio was deduced with such concentrates from the monkey, dog, sheep, cow and rabbit as was also the case with the products employing Al-Susp and amine pre-treatment. Although the results have been similar with human concentrates, some discrepancies were noted earlier with SI prepared from the intestines of children who succumbed to various diseases, including cystic fibrosis of the pancreas (Gibbs and Gershbein, 1950). In the horse, elk and the rat, species without gall bladder, CCK was unequivocally present and the ratio of secretin to CCK in SI approached unity except for the elk. As both secretin and CCK activities were relatively low in many horse SI concentrates, even in those prepared from intestinal strips removed within 30 min after slaughter, the institution of such procedure as pretreatment with chloroform and Al-Susp, led to enrichment. The diminished levels of CCK often encountered in the horse extracts and the inadequate assay at that time, might have contributed to the observation of Ivy in 1934 that CCK appeared to be absent in this species. As attractive as an hypothesis might have seemed that CCK does not occur in animals without gall bladder, this agent must play a more generalized role in smooth muscle contractility, affecting other organs in addition to the unique action on the circular fibers of the gall bladder (Gershbein *et al.*, 1953; Gershbein and Denton, 1967; Denton and Gershbein, 1967).

Acknowledgments

The author is indebted to the management staffs of various packing plants in Chicago and especially to Dr. Stanley Hier of the Wilson Laboratories, for availing him the facilities for beef, pork and lamb intestinal collections, to the

Ken-L Ration Division of Quaker Oats Co., Rockford, Illinois for the horse intestinal strips and to several hunters for the elk A-ppt shipped from Montana.

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Manuscript received April 14, 1967.

TABLE 1.—Isolation of Salt (SA) and SI Concentrates from Porcine Intestinal Extract, Filt I

Concentrate	Mode of Filt I Treatment[a]	Aliquot, ml	SI			Activity per 1 Filt I u x 10^{-3}	
			Yield, mg	Assay, u/mg			
				Secretin	CCK	Secretin	CCK
Series B (4 kg A-ppt; Filt I: 11.87 l)							
SI-47	Usual procedure[b]	2,600	5,680	3.6	3.6	7.9	7.9
SI-48	Filtration after chilling for 3 hr[c]	400	*A* 870 *B* 162	3.5 0.60	3.5 0.58	7.6 0.24	7.6 0.24
SI-49	TCA addition to a level of 25%	400	942	2.5	2.4	5.9	5.7
SI-50	Shaking with chloroform	3,560	6,260	2.6	4.0	4.6	7.0
SI-54	Addition of Al-Susp (400 ml)	400	188	9.7	9.5	4.6	4.5
SI-55	Batch treatment with 12 g Lloyds' Reagent	400	429	0.067	<0.30	.....	.....
SI-56	Batch treatment with Zeo-Karb H (23 g)	400	876	3.3	3.3	7.2	7.2
SI-57	Batch treatment with IR-100 (23 g)	400	954	3.4	3.4	8.1	8.1
Series C (441 g A-ppt; Filt I: 945 ml)							
SA-R6	Salt precipitate with excess acetone	100	5,274	0.052	0.050	2.6	2.6
SI-R8	Usual SI procedure	100	196	2.0	2.0	3.9	3.9
SI-R9	Chloroform	100	156	1.4	2.8	2.2	4.4
SI-R10	Treatment with Al-Susp (200 ml)[c]	200	*A* 65 *B* 45	4.9 1.1	5.1 2.7	1.6 0.25	1.6 0.61

TABLE 1.—Isolation of Salt (SA) and SI Concentrates from Porcine Intestinal Extract (Cont'd)

Concentrate	Mode of Filt I Treatment	Aliquot, ml	SI			Activity per l Filt I u x 10^{-3}	
			Yield, mg	Assay, u/mg			
				Secretin	CCK	Secretin	CCK
Series D (600 g A-ppt; Filt I: 1515 ml)							
SA-78	Excess acetone	100	3,580	0.35	0.35	1.25	1.25
SI-79	Usual SI procedure	200	478	5.6	5.6	1.34	1.34
SI-84	Al-Susp	615[d]	208	16.0	16.0	0.54	0.54
SI-85	Al-Susp treatment followed by deproteinization with chloroform	195[d]	87	12.6	12.6	0.56	0.56
SI-88	Chloroform	94[d]	102	4.0	6.8	0.43	0.74
SI-90	Deproteinization with chloroform followed by Al-Susp.	306[d]	31	6.3	9.6	0.06	0.10
Series E (997 g A-ppt; Filt I: 1620 ml)							
SA-95	Excess acetone	100	7,280	0.25	0.27	18.2	19.7
SI-96	Usual SI procedure	800	3,095	5.8	5.8	22.4	22.4
SI-97	Passage over column of Deacidite 735 (58 g)	100	277	5.8	5.1	16.1	14.1
SI-98	Column treatment with Permutit S (64 g)	100	251	6.0	5.9	15.1	14.8
SI-99	Column treatment with Permutit according to Folin (28 g)	100	——[e]	.....	.....	.....	.....

a. Unless otherwise stated, TCA was added to Filt I as such or after preliminary processing as indicated to a concentration of 5% and the product chilled for 72 hr prior to filtration of the SI concentrate.

b. SI concentrates were similar in yield and hormonal activity where the period of chilling was 48 hr or when TCA was added to a final level of 10%.

c. The filtrate resulting after removal of the *A* concentrate was again treated with the same volume of TCA solution and the *B* product filtered after chilling for 3 days.

d. Yields are calculated on the basis of the volume of the final Al-Susp or chloroform-deproteinized filtrate.

e. No solid resulted even after prolonged chilling.

TABLE 2.—Distribution of Secretin and CCK in SI Concentrates of Animal Species Without Gall Bladder[a]

No. of Intestinal Strips	Filt I Aliquot, ml	SI		
			Assay, u/mg	
		mg	Secretin	CCK
HORSE				
18 (Filt I: 1000 ml)	250; usual SI procedure	186	<0.1	<0.1
	750; Al-Susp	64	0.50	0.50
22	Chloroform treatment	910	1.2	1.8
12 (Filt I: 650 ml)	150; usual procedure	146	0.78	0.78
	500; Al-Susp	179	2.0	2.0
RAT[b]				
24		22	1.5	1.6
82		134	1.9	1.9
21		24	2.0	2.2
24		38	2.0	2.3
30	Chloroform treatment	33	1.3	2.7
ELK				
2		253	<0.1	0.21

a. Unless otherwise stated, the usual procedure as described in the text was employed in the precipitation of SI.

b. The animals of either sex ranged from 150 to 250 g in weight.

TABLE 3.—Summary of Hormonal Activity Distribution and Recovery of Concentrates Treated with Chloroform, A1-Susp and Aniline

Starting Product	Treatment	Assay, u/mg		Ratio: CCK/ Secretin	Unitage Recovered %	
		Secretin	CCK			
SERIES B						
Filt I	Usual SI procedure (SI-47)	3.6	3.6	1.0	.....	.....
SI-47	Conventional aniline treatment (VII-47)	9.1	9.1	1.0	48[b]	48[b]
Filt I	Chloroform (SI-50)	2.5	4.0	1.6	58	90
SI-50	Aniline treatment (VII-50-N)	3.0	11.0	3.7	20[b]	45[b]
SI-50	Aqueous residue after aniline removal treated with Al-Susp (VII-50-CR)	2.3	5.9	2.6	11[b]	18[b]
SERIES D						
Filt I	Usual (SI-79)	5.6	5.6	1.0	.....	.....
Filt I	Al-Susp (SI-84)	16.0	16.0	1.0	41	41
Filt I	Al-Susp followed by chloroform (SI-85)	12.6	12.6	1.0	42	42
Filt I	Chloroform (SI-88)	4.0	6.8	1.7	32	55
Filt I	Chloroform followed by Al-Susp (SI-90)	6.3	9.6	1.5	4	7

a. As compared to that of the respective conventional SI.
b. Unitage recovered based on the starting SI.

WOODY VEGETATION SURVEY OF SARGENTS WOODS, COLES COUNTY, ILLINOIS

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ABSTRACT.—An inventory of the woody vegetation of Sargents Woods, Coles County, Illinois, shows that the present stand is composed of 139 stems per acre (above 4″ d.b.h.) with a basal area of 90 square feet per acre. Thirty-seven woody species are present on the site, with white oak being the leading dominant. White ash, red oak, shagback hickory, black oak, and slippery elm followed in order of Importance Value Index. Three broad cover types are recognized on the woodlot. These are a mixed hardwood region, comprising most of the woods, which is dominated by various species of oaks and hickories; a small region dominated by sugar maple; and three small disturbed areas, one formed by recent cutting and two by past home sites.

Sargents Woods is a 25-acre woodlot located about 12 miles southeast of Charleston, Coles County, Illinois, and represents a remnant of a much larger forest that once occupied most of the Shelbyville Moraine. This woodlot was once owned by Paul Sargent, a local artist and amateur naturalist, who maintained the woods in a natural state until his death in 1946. At that time the woods was deeded to the Sam Sargent family, who owns the land at the present time.

The woods has not been grazed during its ownership by the Sargent family and the only major disturbance occurred in 1948, when about 172 oaks were cut for lumber. Some of these trees were scattered throughout the woodlot, but a large number were removed from a small area on the south side of the woods. Many of the stumps are still evident and most of the trees averaged 1 to 2 feet in diameter.

The only previous ecological work in the woodlot was conducted by various ecology classes from Eastern Illinois University that made a few line-transects through the woods. The first complete inventory of the vegetation was done in 1966, and the results are presented in this paper. Results of a similar study in Baber Woods, another remnant of a forest located on the Shelbyville Moraine, were reported previously by McClain and Ebinger 1968. The taxonomic nomenclature used in this paper follows that of Jones (1963).

DESCRIPTION OF THE WOODLOT

Sargents Woods is located in the SW¼, NW¼ of Section 11, T11N, R 10E, Coles County, Illinois, which is at the southern edge of the Shelbyville Moraine that traverses this part of the state in an east-west direction. The topography is gently rolling, with a maximum difference in elevation of about 40 feet. The woodlot is well drained by two small, intermittent streams which extend in an east-west direction through the woods. In a few places these streams

have cut steep banks 30 feet tall. Between the streams is a relatively flat ridge. A farm road extends along the ridge to the southeast corner of the woods where the Sargent house is located.

Method of Study

The entire woodlot was divided into 50 meter square blocks (0.61776 acre), and the number, size, and species of trees were recorded in each. The diameter of all trees having a d.b.h. of more than 4 inches was recorded to the nearest 1/10 inch. Dead-standing and dead-downed trees were also measured and identified when possible. The Importance Value Index (IVI) was then calculated from the actual data to provide a better basis of comparison of the various species. As used here, the determination of the IVI follows the procedures outlined by McIntosh (1957) and later by Boggess (1964), in which the IVI is the sum of the relative density $\left(\frac{\textit{number of individuals of a species}}{\textit{number of individuals of all species}} \times 100\right)$ and of the relative dominance $\left(\frac{\textit{Basal area of a species}}{\textit{total basal area of all species}} \times 100\right)$. The IVI was also calculated for each plot to determine if different vegetation zones existed in the woods.

In each 50 meter block, four 1/100 and 1/1000 acre nested, circular quadrats were randomly located. The saplings (1 to 4 inches d.b.h.) were tallied on the larger plot and the seedlings on the smaller. The seedlings were divided into those under one foot in height and those over one foot in height but less than one inch in diameter.

Results and Discussion

A total of 37 woody species were encountered on the woodlot, of which 17 were understory trees and shrubs. These species, along with their density and frequency by height or diameter class, appear in Table 1. The species symbols will be used to identify the species in subsequent tables and figures. An additional listing of the 10 leading species encountered, with their relative values, average diameters, number of trees, and basal area per acre broken down into broad diameter classes, are included in Table 2.

Three broad cover types have been recognized on the woodlot (Figure 1). These are: 1) three small disturbed regions, one on the east, one on the south, and the other on the west edge of the woodlot; 2) a mixed hardwood region, comprising most of the woods, in which various species of oaks and hickories are dominant, and 3) a sugar maple dominated region. In the three disturbed areas, the important species are white ash, slippery elm, and black walnut. In the mixed hardwood region, white oak is the leading dominant and usually has an IVI per

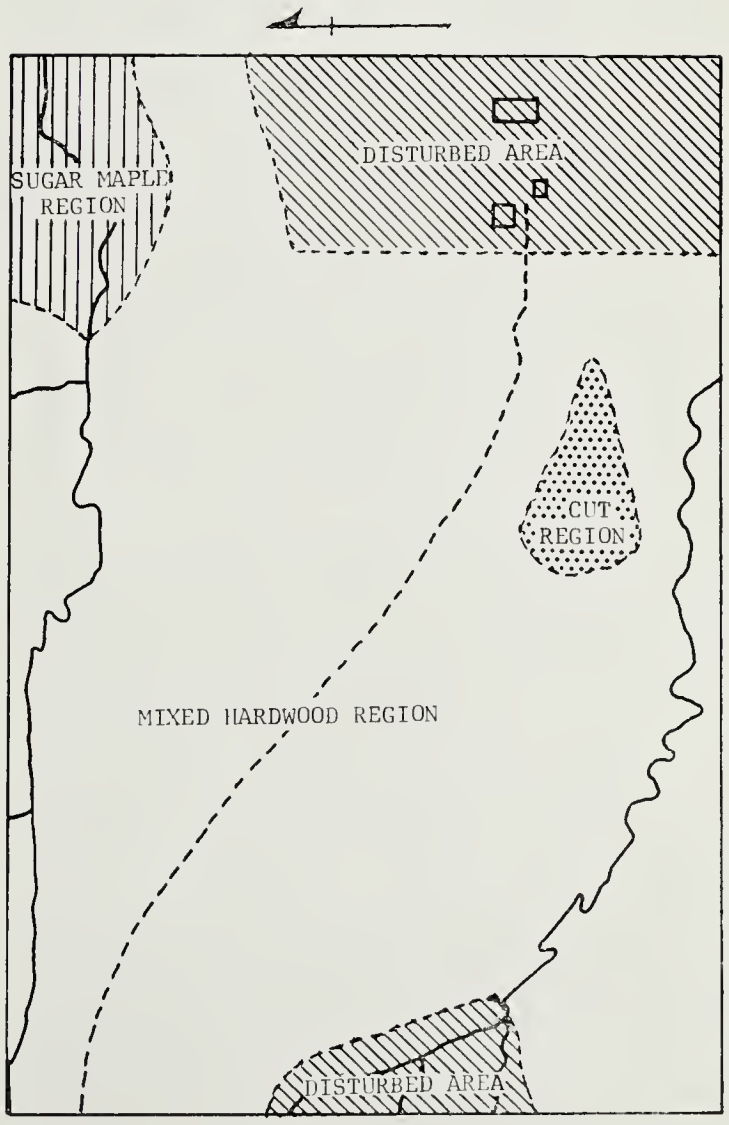

Figure 1.—Map showing the distribution of the broad cover types in Sargents Woods.

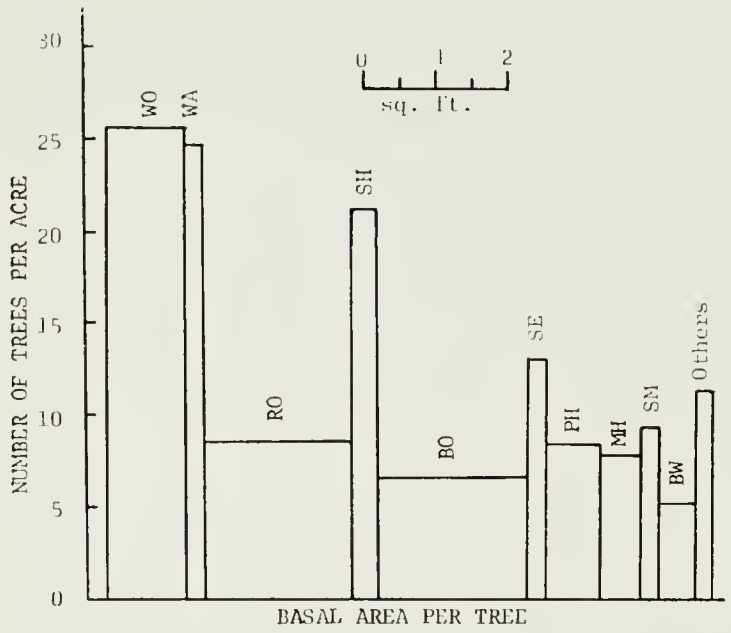

FIGURE 2.—Number of trees per acre and the average basal area per tree for the 10 leading species, and for all other species combined.

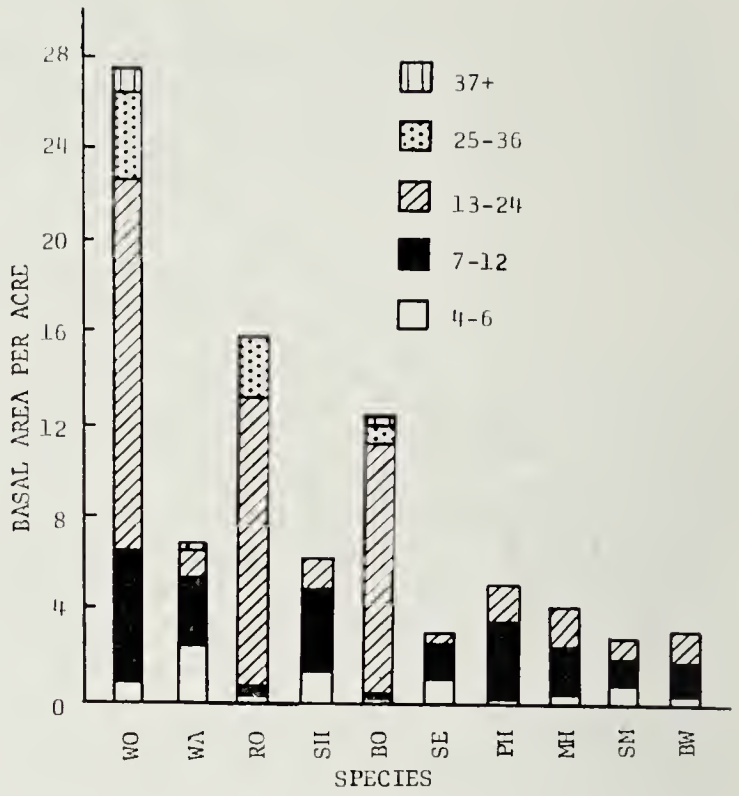

FIGURE 3.—Total basal area and the basal area by diameter classes for the 10 leading species.

plot of more than 40 (maximum possible value, 200). Other important species in this area are white ash, red oak, shagbark hickory, black oak, pignut and mockernut hickory. The sugar maple dominated region occurs in a moist, low area in the extreme northeast corner of the stand.

The present stand composition is shown in Figure 2, which includes the average number of trees per acre and the average basal area per tree for the 10 leading species, and for all other species combined. In this figure, the average basal area per tree is represented by the area included in each bar of the graph (Vestal, 1953). As a further representation of stand structure, the total basal area and the basal area in five broad diameter classes for the 10 dominant species are shown in Figure 3.

Of the woody species encountered in Sargents Woods, white oak has the highest IVI. It comprises about one-fifth of the total number of individuals and almost one-third of the stand basal area. It is well represented throughout the stand, with frequencies ranging from 28 percent in the seedling less than 1 foot tall to 100 percent for the larger trees. On each plot, its IVI ranges from 15 to 88, with an average of 48.78, nearly twice as great as the value for the next important species. White oak ranks fifth in the number of seedlings per acre, eleventh in saplings, and fifth in the 4-6 inch diameter class. In all other broad diameter classes, it ranks first in trees per acre. Its importance is mainly due to the large size of individual trees. The average diameter for this species is 14.0 inches, and the largest tree in the woodlot is a 43-inch white oak.

White ash ranks second in IVI because of its high relative density. It is first in the number of seedlings with over 3,000 per acre and dominates the 4-6 inch diameter class. The number of larger trees is small as indicated by its average diameter of 7.1 inches (Table 2) and only one tree was found with a diameter ex-

ceeding 20 inches. The highest concentration of this species occurs in the small disturbed areas along the east and west edges of the woodlot. In these areas its IVI per plot sometimes exceeds 90. Smaller diameter trees of this species are well distributed throughout the woodlot and it was found in every plot. Except for in the disturbed area, its IVI per plot is about 20.

The importance of red and black oak (third and fifth in IVI) is due mostly to the large size of individual trees as indicated by their diameters of 18.7 and 19.3 inches, respectively. Both species rank very low in seedlings and saplings per acre and average less than 1 tree per acre in both the 4-6 and 7-12 diameter classes. They rank second and third in tress per acre in the 13-24 and 25-36 inch diameter classes and black oak is the only species other than white oak which has trees in the 37 inch and higher diameter class. Both species occur in nearly all plots and their IVI's on some plots exceed that of white oak.

Four species of hickory are found in the woodlot. Shagbark hickory ranks fourth in IVI mainly because of the large number of small diameter trees that are scattered throughout the woods. This species ranks second in the number of trees per acre in the 4-6 and 7-12 inch diameter classes and is found in every plot. Pignut hickory and mockernut hickory (seventh and eighth in IVI) are represented in the woods by larger diameter trees as indicated by their average diameters of 11.0 and 9.9 inches, respectively. Both species are poorly represented in the sapling and seedling categories as well as in the 4-6 inch diameter class. Also, bitternut hickory is occasionally found through the woods, but is only fifteenth in IVI. The numerous shagbark hickories in the 4-6 and 7-12 inch diameter classes is a possible indication that this species is able to reproduce more readily under forest conditions than the other three species of hickories. Similar results were obtained by McClain and Ebinger (1968) in their study of Baber Woods, which is located on the Shelbyville Moraine.

Sugar maple (ninth in IVI) is poorly represented throughout most of the stand. In the mixed hardwood and disturbed areas a few trees were found in most of the plots, but these chiefly belonged to the 4-6 inch diameter class. In the sugar maple dominated region in the extreme northeast corner of the stand, larger trees of this species occur. Here the IVI for sugar maple exceeds 45, and a few trees exceed 20 inches in diameter. In this region, ironwood also is an important stand component and has an IVI of 25 per plot. In other parts of the woodlot this species is rarely found. This sugar maple dominated region follows along the stream on the north edge of the stand and from here the size and number of sugar maple rapidly decrease through the remainder of the woodlot. It appears that this species has become well established along this small stream and may spread through the entire area. A similar situation was found by McClain and Ebinger (1968) in their study of Baber Woods.

The relative importance of the 10 leading species is illustrated in Table 3, which gives the number of plots

on which each species had the highest IVI, or ranked second, third, or fourth, or less.

The understory is dominated by poison ivy which averages more than 5,000 stems per acre. In the wetter areas of the woods, particularly at the edge of streams, gooseberry *(Ribes missouriense)* is very common. Black haw, hazlenut, and wild hydrangea are also common along with the seedlings and saplings of various tree species.

The number of dead-standing and dead-downed trees is not an important feature of the woodlot. The total mortality of all species is only 8.8 trees per acre with a basal area of 5.5 square feet per acre. Dead elms average 3.2 trees per acre with a basal area of 1.1 square feet per acre. This is extremely low, particularly when compared with the woods to the north of the Shelbyville Moraine. In Trelease Woods (Boggess, 1964), dead elms averaged 27.6 trees per acre with a basal area of 44.9 square feet per acre, while in Burgner Acres (Blackmore and Ebinger, 1967), dead elms averaged 15.1 trees per acre and had a basal area of 14.8 square feet. Nearly all of the dead elms in Sargents Woods are in the 4-6 and 7-12 inch diameter classes and none were found with a diameter in excess of 18 inches. The relatively few large dead elms suggest that this species has never been an important stand component. Even with the large numbers of seedlings and saplings found (Table 1) the high mortality of elms due to phloem necrosis and the Dutch elm disease will probably continue to limit its importance in the woodlot. In contrast to elm, black oak mortality was largely among the veteran trees. These were scattered throughout the woods and averaged 1.3 trees per acre with an average basal area of 1.9 square feet per acre. Cottonwood is not represented by living trees in the woods, but two dead specimens were found along one of the streams. These were 32 and 47 inches in diameter, giving it a total basal area of 17.6 square feet in the woods. The mortality of the other species is extremely low and very few large trees were found.

In 1948, about 172 trees were cut out of the woodlot. Many of these were scattered along both sides of the farm road that extends along the ridge to the Sargent house. The remainder were cut from a small area on the south side of the woods (Figure 1). Many of the stumps are still present and it appears that most, if not all of the trees cut were species of oak. Measurements of the stumps indicate that at least 449 square feet of basal area was removed from the woodlot. This past cutting has had some effect on the present composition of the forest. Ash, and to some extent elm, have taken advantage of these canopy openings which is probably the reason for their high IVI in the woods.

Acknowledgments

The author is grateful to the Eastern Illinois University Research Committee for support to complete this project and to W. R. Boggess and J. W. Geis, University of Illinois, for their advice and suggestions concerning the survey and the manuscript.

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Manuscript received June 29, 1967.

TABLE 1.—Density Per Acre and Frequency of Woody Species by Height or Diameter Class. The species symbol will be used to identify species in subsequent tables and figures.

Scientific Name	Common Name	Symbol	Height Class					Diameter Class			
			Density			Frequency %		1″-4″		4″+	
			<1′	>1′<1″ dbh	Total	<1′	>1′<1″ dbh	Density	Frequency %	Density	Frequency %
Quercus alba L.	White oak	WO	513	237	750	28.8	17.9	7	7.1	25.57	100
Fraxinus americana L.	White Ash	WA	3,949	865	4,814	78.2	41.7	48	27.6	24.53	100
Quercus rubra L.	Red oak	RO	109	160	269	9.0	14.1	3	3.2	8.24	91
Carya ovata (Mill.) K. Koch	Shabgark hickory	SH	468	231	699	32.7	19.2	46	34.6	21.09	100
Quercus velutina Lam.	Black oak	BO	173	160	333	15.4	14.1	4	3.2	6.03	83
Ulmus rubra Muhl.	Slippery elm	SE	1,333	1,500	2,833	53.2	59.0	106	47.4	12.75	100
Carya glabra (Mill.) Sweet	Pignut hickory	PH	58	205	263	5.1	12.8	19	9.6	8.04	82
Carya tomentosa (Poir.) Nutt.	Mockernut hickory	MH	26	115	141	2.6	10.9	21	16.7	7.70	85
Acer saccharum Marsh.	Sugar maple	SM	244	199	443	10.9	15.4	142	49.4	9.03	88
Juglans nigra L.	Black walnut	BW	.....	19	19	.....	1.9	1	1.3	5.20	76
Ostrya virginiana (Mill.) K. Koch	Ironwood	Ir	987	96	1,083	10.3	7.1	17	9.0	2.80	21
Morus rubra L.	Red mulberry	RM	6	71	77	0.6	4.5	6	6.4	2.11	58
Celtis occidentalis L.	Hackberry	Ha	19	32	51	1.9	3.2	6	3.2	1.67	36
Prunus serotina Ehrh.	Black cherry	BC	244	179	423	16.7	16.0	10	6.4	1.37	52
Carya cordiformis (Wang.) K. Koch	Bitternut hickory	BH	45	103	148	1.9	9.6	12	7.1	0.59	30
Sassafras albidum (Nutt.) Nees	Sassafras	Sa	77	45	122	4.5	3.8	7	4.5	0.44	18
Cercis candadensis L.	Red bud	RB	590	269	859	23.7	13.5	61	24.4	0.59	24
Gleditsia triacanthos L.	Honey locust	HL	.....	.....	.....	.....	.....	.....	.....	0.15	3
Quercus imbricaria Michx.	Shingle oak	SO	38	13	51	3.8	0.6	.....	.....	0.39	6

TABLE 1. (Continued)

Scientific Name	Common Name	Symbol	Height Class					Diameter Class			
			Density			Frequency %		1"-4"		4" +	
			<1'	>1'<1" dbh	Total	<1'	>1'<1" dbh	Density	Frequency %	Density	Frequency %
Gymnocladus dioicus (L.) K. Koch	Ky. coffee tree	KC						1	0.6	0.34	3
Tilia americana L.	Basswood	Ba								0.20	3
Quercus macrocarpa Michx.	Bur oak	Bu								0.10	3
Quercus muhlenbergii Engelm.	Chinquapin oak	CO	6		6	0.6				0.10	6
Morus alba L.	White mulberry	WM						1	0.6	0.10	3
Maclura pomifera (Raf.) Schneid.	Osage orange	OO		6	6		0.6			0.05	3
Cornus florida L.	Flowering dogwood	FD						1	0.6	0.05	3
Crataegus crusgalli L.	Cockspur thorn	CT		6	6		0.6			0.05	3
Rhus radicans L.	Poison ivy	PI	1,481	4,506	5,987	48.7	53.9				
Viburnum prunifolium L.	Blackhaw	Bl	38	109	147	3.8	3.8	4	1.9		
Corylus americana Walt.	Hazelnut	Hz	13	128	141	1.3	8.3				
Ribes missouriense Nutt.	Gooseberry	Go	64	51	115	5.1	3.8				
Celastrus scandens L.	Bittersweet	Bi	32		32	2.6					
Hydrangea arborescens L.	Wild hydrangea	WH		77	77		0.6				
Crataegus mollis (T.&G.) Scheele	Red haw	RH	13		13	0.6					
Diospyros virginiana L.	Persimmon	Pe	26	19	45	1.9	1.9				
Asimina triloba (L.) Dunal	Pawpaw	Pa		6	6		0.6				
Ceanothus americanus L.	New Jersey tea	NJ		6	6		0.6				
Total			10,552	9,413	19,965			523		139.28	

TABLE 2.—Number of Trees, Basal Area Per Acre, Importance Value Index and Average Diameter for Leading Dominants.

Species	4-6		7-12		13-24		25-36		37 +		Total		Percent Total		I.V.I.	Av. Diam., In.
	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	No.	B.A.	Rel. Den.	Rel. Dom.		
WO	4.12	0.62	9.76	5.66	10.79	16.49	0.78	3.70	0.10	0.88	25.55	27.35	18.34	30.44	48.78	14.0
WA	16.04	2.31	7.51	3.13	0.93	1.16	0.05	0.18	.....	.....	24.53	6.78	17.62	7.55	25.17	7.1
RO	0.39	0.06	0.74	0.49	6.62	13.22	0.49	2.02	.....	.....	8.24	15.79	5.92	17.57	23.49	18.7
SH	12.51	1.83	7.51	3.63	1.08	1.22	.....	.....	.....	.....	21.10	6.68	15.15	7.43	22.58	7.6
BO	0.29	0.05	0.34	0.23	5.10	10.57	0.25	0.95	0.05	0.45	6.03	12.25	4.33	13.63	17.96	19.3
SE	8.88	1.22	3.58	1.47	0.30	0.36	.....	.....	.....	.....	12.76	3.05	9.16	3.39	12.55	6.6
PH	0.74	0.13	5.69	3.24	1.62	1.94	.....	.....	.....	.....	8.05	5.31	5.78	5.91	11.69	11.0
MH	2.40	0.39	4.02	2.09	1.28	1.67	.....	.....	.....	.....	7.70	4.15	5.53	4.62	10.15	9.9
SM	6.33	0.85	2.16	0.85	0.54	0.78	.....	.....	.....	.....	9.03	2.48	6.48	2.76	9.24	7.1
BW	1.57	0.26	2.70	1.22	0.93	1.43	.....	.....	.....	.....	5.20	2.91	3.73	3.24	6.97	10.1
Others	7.85	1.11	2.80	1.16	0.39	0.65	0.05	0.19	.....	.....	11.09	3.11	7.96	3.46	11.42	8.0
Total	61.12	8.83	46.81	23.17	29.58	49.49	1.62	7.04	0.15	1.33	139.28	89.86	100.00	100.00	200.00	.....

TABLE 3.—Importance Value Index, by Numbers of Plots for the Ten Leading Species.

Species	IVI Rank by Numbers of Plots			
	1st	2nd	3rd	4th or Below
WO	19	8	4	2
WA	4	4	9	16
RO	5	2	7	16
SH	1	10	3	19
BO	3	3	1	22
SE	.....	1	4	28
PH	.....	2	1	24
MH	.....	1	1	26
SM	1	.....	2	26
BW	.....	2	.....	23

THE FUNGITOXICITY OF THIRAM, CAPTAN, AND CHEMAGRO 2635 IN RELATION TO ORGANIC MATTER AND CLAY CONTENT OF SOIL

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Abstract—Three Illinois soils differing in clay and organic matter content were used to determine the effect of clay and organic matter on the retention of the fungitoxicants, thiram, captan, and Chemagro 2635. Soil plugs treated with these chemicals at 25 to 100 ppm were placed in glass rings on potato dextrose agar seeded with *Diplodia zeae*. The size of the zone of inhibition around each soil plug indicated that thiram and Chemagro 2635 were released more readily from the soil with the lowest water holding capacity and the least organic matter. No relationship between clay content and retentivity was found. There was no real difference in the retention of captan among any of the three soils.

Variation in the performance of soil fungitoxicants from one experiment to another seems to be the rule rather than the exception. Inconsistencies have been attributed in part to sorption of the toxicants by one or more of the soil components (Kreutzer, 1963).

The influence of various soil components on volatile fungitoxicants, nematocides, and insecticides have been studied by a number of investigators. Munnecke and Ferguson (1953) reported that the amount of sorption of methyl bromide is directly proportional to the organic matter content of the soil. According to Siegal *et al* (1957) the diffusion of 1,3-dichloropropene and 1,2-dibromoethane in soils decreases as soil organic matter increases. Call (1957) and Wade (1954) found the sorption of ethylene dibromide in soil could be directly correlated with organic matter and less closely with clay content. Lichtenstein and Schulz (1959) reported that chlorinated hydrocarbon insecticides persisted longer in muck soils than in soils lower in organic matter. Rushdi and Jeffers (1956) claimed that an increase in the type and amount of organic matter or in the clay content of the soil decreased the activity of fungicides against *Rhizoctonia solani* Kuhn. Gray (1962) reported that Vapam (sodium N-methyldithiocarbamate) was completely degraded in heavy clay and peat soils in one hour, but degradation was slower with a decrease in the clay and peat content. Allen and Raski (1950) suggested that lack of effective control of the sugar beet nematode in California by DD mixture may have been due in part to diffusion through soils "containing clay and these high in organic matter."

The effect of various soil components on non-volatile diffusible fungi-

toxicants has received little attention. Munnecke (1961) found that 13 fungitoxicants, including thiram and captan, penetrated the greatest distance through columns of soils lowest in organic matter. Movement of the chemicals was restricted more by the finer clay fraction than by the coarser fraction. Richardson (1954) found that although thiram persisted in sandy soil to the end of a 46-day test period, it disappeared in compost soil within a week.

The purpose of the present investigation was to study the toxicity of three fungitoxicants in relation to organic matter and clay in three soils chosen for their geometric increase in clay content.

Materials and Methods

Soil. Three Illinois soils differing widely in composition were collected from fields under cultivation in Kankakee, Ogle, and Champaign counties (Table 1). The predominant clay type in all three was montmorillonite.

The soils were passed through a ⅛ inch mesh screen and spread out on paper to air-dry. The Kankakee and Ogle soils were adjusted to pH 6.0, which was the pH of the Champaign soil, with dilute sulfuric acid prior to steam sterilization. Three hundred grams of soil were placed in a 500 ml Erlenmeyer flask which was plugged with cotton and steam sterilized for 30 minutes at 15 psi. There were 10 flasks of each of the three kinds of soil.

To determine the water holding capacity of each soil, 400 g of air-dried, autoclaved soil were put in a Lucite plastic tube 1¾ inches (i.d.) by 12 inches long weighing 377 g. The soil was packed by gently tapping the column vertically six times on the table. Water was added and allowed to move through the soil until it dripped from the bottom of the column. After 24 hours the column and soil were weighed again with the difference in weight representing the moisture content.

Fungitoxicants. Three fungitoxicants were used: Captan 50W - 50% captan (N-trichloromethylthio) - 4-cyclohexene-1,2-dicarboximide; Arasan 75 - 75% thiram (bis (dimethylthiocarbamoyl) disulfide); Chemagro 2635 (80% 1,2,4-trichloro-3,5-dinitrobenzene and 20% 1,2,3-trichloro-4,6-dinitrobenzene). The three chemicals were selected because they gave well-defined, measurable zones of inhibition of the test fungus at the concentrations to be used. The three toxicants were employed at 25 and 100 ppm of active ingredients. Fifty ppm was also used, but results are not given here. The required amount of the toxicant and 300 g of sterilized soil from the 500 ml flask were mixed in a Patterson-Kelly twin-shell blender for 15 minutes and returned to the flask. Sterile de-ionized water was added to each soil-fungitoxicant mixture to bring it to its respective water holding capacity.

Fungitoxicant assay. The influence of the soils on the activity of captan, thiram, and Chemagro 2635 was determined by using a modification of the technique described by Munnecke (1958). Potato dextrose agar (PDA) plates were seeded with the test fungus, *Diplodia zeae* (Schw.) Lev., by chopping up two 12-day-old Petri dish cultures in a Waring Blendor for 30 seconds, mixing thoroughly with PDA which had been cooled to 48° C., and then pouring 12 ml into each Petri dish. Soil samples were taken from each of the flasks by pushing a glass tube 11 mm in diameter into the soil. The soil was pushed from the tube with a glass rod into a glass ring 12 mm in height by 11 mm in diameter. The ring containing the soil-fungitoxicant mixture was placed in the center of a Petri dish containing PDA seeded with the test fungus (Figure 1). There were six replications of each treatment. Control plates were prepared by placing rings of autoclaved soil without the fungitoxicant on a seeded agar plate.

The diameter of the inhibition zone was measured at the end of 1, 2, 4, 7, 10, and 14 days. The diameter of the zone of inhibition included the diameter of the ring containing the soil-fungitoxicant mixture.

Results and Discussions

Thiram and Chemagro 2635 were released more readily from the Ogle soil than from the Champaign or Kankakee soils as evidenced by

FIGURE 1.—Typical zone of inhibition of *Diplodia zeae* resulting from diffusion of thiram from soil into potato-dextrose-agar.

larger zones of inhibition from these chemicals (Figures 2 and 3). There appeared to be no difference in the retention of captan among the three soils (Figure 4). The concentration

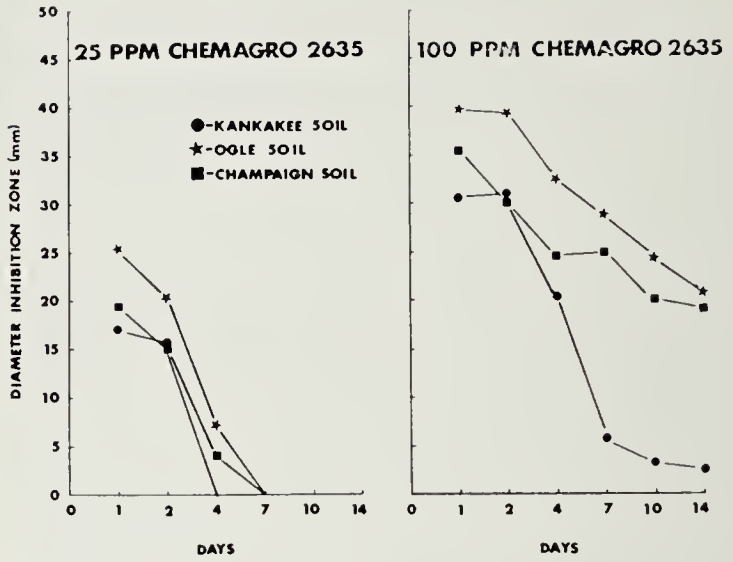

FIGURE 3.—Inhibition of growth of *Diplodia zeae* by Chemagro 2635 as influenced by three soil types.

of thiram and Chemagro 2635 affected the persistence and size of the zone of inhibition, but that of captan apparently did not. It is not known whether this phenomenon with captan is a function of degradation in the soil or of some other factor. It might be assumed from the larger zones of inhibition that thiram showed more specificity for *Diplodia zeae* than the other two toxicants. However, since this technique depends upon the diffusibility of the fungitoxicant through agar and subsequent killing or inhibiting

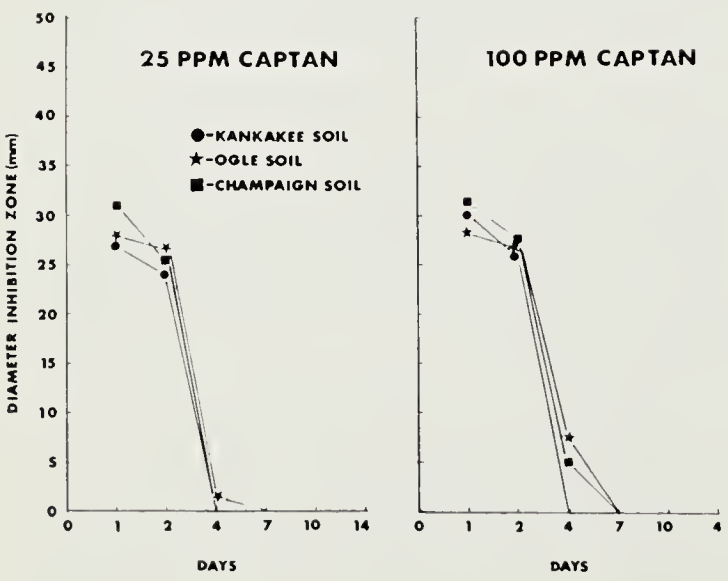

FIGURE 4.—Inhibition of growth of *Diplodia zeae* by captan as influenced by three soil types.

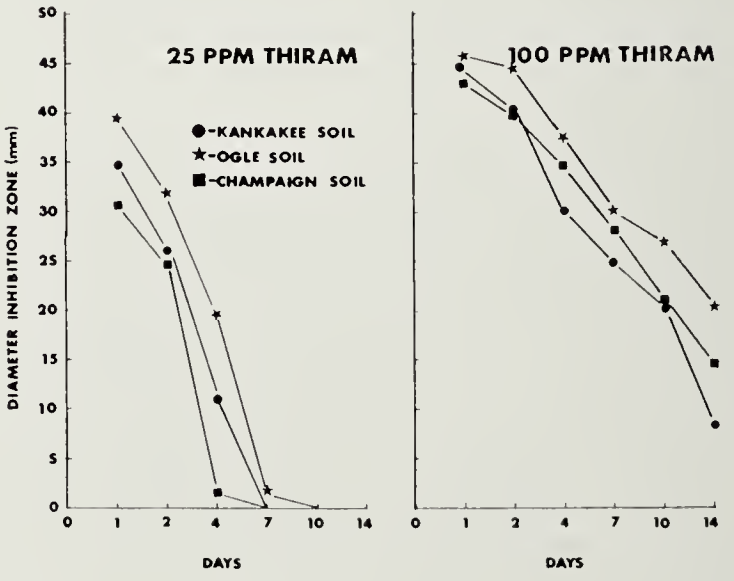

FIGURE 2.—Inhibition of growth of *Diplodia zeae* by thiram as influenced by three soil types.

TABLE 1.—Characteristics of the three soil types used.

Soil Type and Location	% Clay	% Sand	% Silt	% Organic Matter	pH[a]	Exchange[b] Capacity	% Water Holding Capacity
Maumee fine sandy loam..... (Kankakee County)	9.8	75.2	15.0	4.3	7.0	10.3	30.0
Brenton silt loam..... (Ogle County)	18.7	26.7	54.6	3.2	7.2	18.3	25.0
Drummer silty clay loam..... (Champaign County)	36.7	16.6	46.7	4.9	6.0	32.4	28.5

a Before adjustment.
b Milliequivalents per 100 grams.

of the test organism, it cannot be used to compare the effectiveness of fungitoxicants (Munnecke, 1958). The rate of disappearance of the zones was similar for thiram and Chemagro 2635 but different for captan.

The greater relative release of the two fungitoxicants from the Ogle soil was apparently related to the lower amount of organic matter in this soil (Table 1). Organic matter influences soil properties far out of proportion to the amount present and can account for up to 90 percent of the adsorptive capacity of soil (Buckman and Brady, 1960). There was no evidence of any relationship between retentivity and clay content as claimed by a few investigators. If chemical toxicants are absorbed more readily on soils relatively higher in clay content than on those lower in clay (Allen and Raski, 1950; Gray, 1962; Rushdi and Jeffers, 1956), one could assume the chemicals mixed with the Kanakee soil, the lowest in clay, would have shown larger zones of inhibition.

Moisture together with organic matter is important in determining the degree of retention of toxicants in soil. The lower water holding capacity of the Ogle soil may have been responsible for the greater release of thiram and Chemagro 2635. The organic matter content of a soil is critical at its water holding capacity in retention of toxicants because soils rich in organic matter contain more extensive films of water on which toxicants can be sorbed (Birchfield, 1960). In addition, an increase in organic matter also increases the possibility of both covalent bonding and solution of toxicants in solvents of an organic nature (Kreutzer, 1960). These two factors could account for a difference in performance of toxicants from one soil to another. Nonvolatile materials or compounds with a low vapor pressure, such as those used in the present investigation, would not be lost from soil as rapidly as volatile materials and thus would be more subject to chemical change in wet soils rich in organic matter.

Acknowledgements

Based on a portion of an M. S. thesis by the senior author.

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Manuscript received May 16, 1967

AN ASSESSMENT OF CHANGES IN THE FISH FAUNA OF TWO ILLINOIS RIVERS AND ITS BEARING ON THEIR FUTURE

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ABSTRACT. Based on a comparison of fish collections taken prior to 1901 and between 1956-1966 in the Vermilion and Embarras River drainages of east-central Illinois, evidence is assembled to show one species extirpated and 20 decimated in the two systems. Analysis of factors probably responsible reveals that the principal ones are alteration of the physical habitat, e. g. rate of flow and bottom type; increase in the amount of suspended silt and subsequent loss of aquatic vegetation; and reduction in stream size and that these factors have probably been of greater significance than pollution *per se* in producing long-term changes in populations of stream fishes. Erection of dams and creation of artificial reservoirs have contributed most dramatically to the observed changes in the fish fauna of the two basins. An artificial reservoir contemplated for the Middle Fork will eradicate the only Illinois population of the bluebreast darter, and future reservoirs at other sites on the Embarras and Vermilion rivers could endanger Illinois populations of the harlequin darter, eastern sand darter, dusky darter, and possibly a few other species of small fishes.

The current arousal of public interest in pollution and related phenomena in our streams and local interest in the future of the Wabash River basin prompt publication of data that provide an opportunity for an objective assessment of changes in the fish fauna of two relatively well-studied rivers in east-central Illinois. The results of this assessment are instructive in that the principal factors producing changes are identified and can thus be given consideration in the future development of the two basins.

Collections of fishes from the Vermilion (of the Wabash drainage) and nearby Embarras rivers have been made over a period of nearly 70 years. These collections supply documentation for several pronounced changes in species composition, most notably the decimation of certain fishes, and permit an appraisal of the several factors that may be responsible for these changes. They further provide an opportunity to scrutinize the role that pollution has played and, as will be shown, they suggest that other factors have been of greater importance than pollution in producing long-term changes in these populations of stream fishes.

Although detailed information is not available on the amount of modification of the watersheds and condition of the streams at the time the early collections were made, we can extrapolate to some degree from the observed changes that have accompanied an increase in human

population, land utilization, and industrialization during recent decades. We can also assume that some alteration of the watersheds had occurred and some pollution was present even 70 years ago but that conditions in the two river systems were vastly different from those of today.

The two rivers are tributaries in Illinois of the middle Wabash River (Fig. 1). Both have headwaters in Champaign County, Illinois, and flow in a generally southeasterly direction, emptying into the Wabash River about 100 straight-line miles apart. They have many physical and faunistic similarities and have been subjected to similar alterations and modifications in approximately the same span of time.

Résumé of Changes in the Streams and Their Watersheds

The watersheds of both rivers are primarily agricultural, the two major crops being corn and soy beans. To increase the acreage of tillable land, most of their basins have long been in drainage districts, the principal purposes of which are to drain marshy areas and to hasten runoff of rain water. Because of the amount of silt carried into the stream and that deposited at the mouths of field tiles, repeated dredging, and straightening, and sometimes recanalizing are necessary.

The principal effect of these activities is that the meandering stream with alternating riffle and pool and a variety of habitats is converted into a relatively straight and uniform canal with fewer habitats. Dredging has been particularly intensive in the headwaters and tributaries of the Vermilion River. Canalization has been most intensive in the lower course of the Embarras River; in fact, a total of more than 10 miles of the lower river now occupies a man-made channel.

With accelerated drainage the water table has been lowered, and the streams have become wider, distinctly shallower, and much more variable in size and amount of flow than they were before the establishment of drainage districts and canals (Larimore & Smith 1963). During periods of drought the rivers become quite low, and many of the once permanent tributaries now dry up.

To increase productivity of the soil, considerable quantities of commercial fertilizers and, in recent years, herbicides and insecticides have been applied to fields in the watersheds. These materials, along with silt, are washed into the streams by rain, sharply reducing the water quality.

Grazing animals erode banks of pasture streams, muddy the water by wading in it, and frequetly drop their wastes directly into the streams. Gravel-washing operations contribute substantial amounts of silt.

Chronic pollution from sewage disposal plants is present at several sites in the Vermilion River drainage. Effluents from the Champaign-Urbana plant prohibit the occurrence of fish in the West Branch of the Salt Fork for much of its length. Sewage from the Danville plant pollutes the Vermilion River several miles below the city, and even exposed sand and gravel bars in the area have a heavy coating of sludge.

Severe pollution from plants at Rantoul and the Chanute Air Force Base enters the upper reaches of the East Branch of the Salt Fork, and smaller amounts of pollution are received from other towns within the drainage.

Industrial pollution from manufacturing plants located near Champaign-Urbana and Danville occurs in the headwaters of the West Branch of the Salt Fork and in the lower reaches of the Vermilion River, although its effects are overshadowed by those of effluents from sewage plants in the same areas. Pollution from extensive oil fields in the lower Embarras River basin has been present for many years, but its effects cannot be assessed since collections of fishes prior to the oil boom are not available for study.

Strip-mining operations, usually devastating to aquatic life, have been carried on for many years along the Middle Fork and lower Vermilion River, but there is, surprisingly enough, no evidence that their wastes have affected the fish populations of these two streams.

In recent years accidental discharges of such toxicants as anhydrous ammonia and potassium cyanide have caused fish kills of varying magnitude in the Vermilion and Embarras rivers and some of their tributaries. However, repopulation of the afflicted part of the stream has been remarkably fast in each case known to me, and no permanent damage can be demonstrated.

Dams, intended to insure water supplies for the cities of Danville and Charleston and to provide much-needed recreational space, have been constructed on the lower North Fork of the Vermilion River and the middle Embarras River.

An 8-foot wooden dam, completed in 1894 on the North Fork near Danville, did not create an impoundment, but a more substantial dam, completed in 1914 by a private utility company, resulted in a several-acre reservoir. Another dam, completed in 1925 by the same company, inundated the old dam and created the present Lake Vermilion. This dam initially flooded 775 acres but because of silting the lake has since shrunk to approximately 617 acres (Dawes & Terstriep 1966b: 134).

A dam, completed in 1947 on the Embarras River near Charleston, impounded 400 acres to form the present Lake Charleston, which still occupies about the same acreage but has become appreciably shallower (Dawes & Terstriep 1966a:35).

Several smaller dams, mostly on tributaries in the Embarras River basin, have resulted in small impoundments that have not had demonstrable effects upon the fishes of the parent rivers.

The more salient features in the streams and watersheds that might have produced changes in the fish fauna of the Vermilion and Embarras River systems are shown in Fig. 1.

Methods of Appraisal

Between 1882 and 1901 (mostly in the 3-year period 1899-1901), personnel from the Illinois State Laboratory of Natural History, under the direction of Stephen A. Forbes, made 59 collections of fishes at 54 sites within the Vermilion and Embarras River basins (Fig. 2). Between 1956 and 1966 my associates and I have

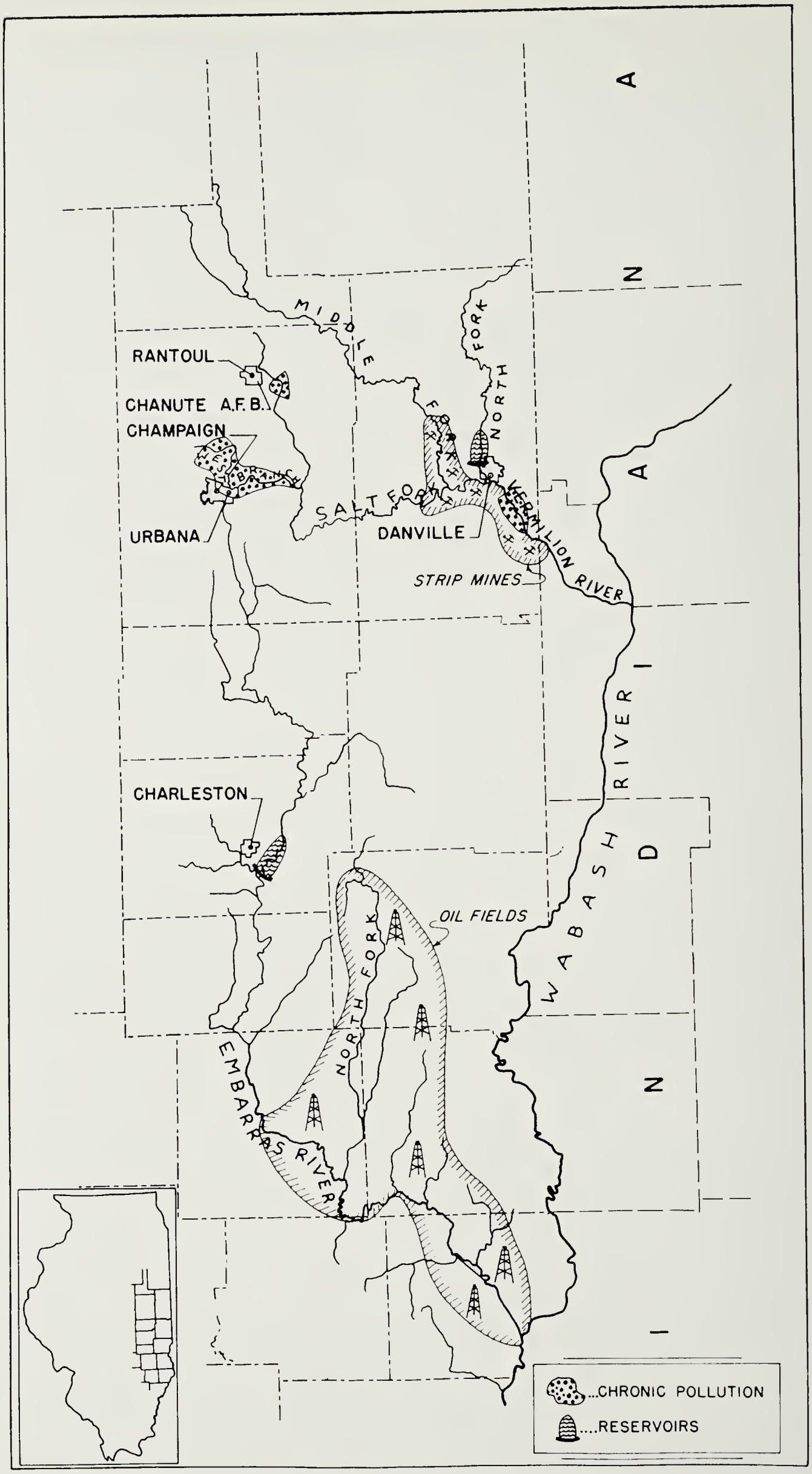


FIGURE 1. — The Vermilion and Embarras River basins, showing the location of cities and streams cited in the text and some areas of major modification of streams and watersheds. The inset map indicates the position of these basins in the state of Illinois.

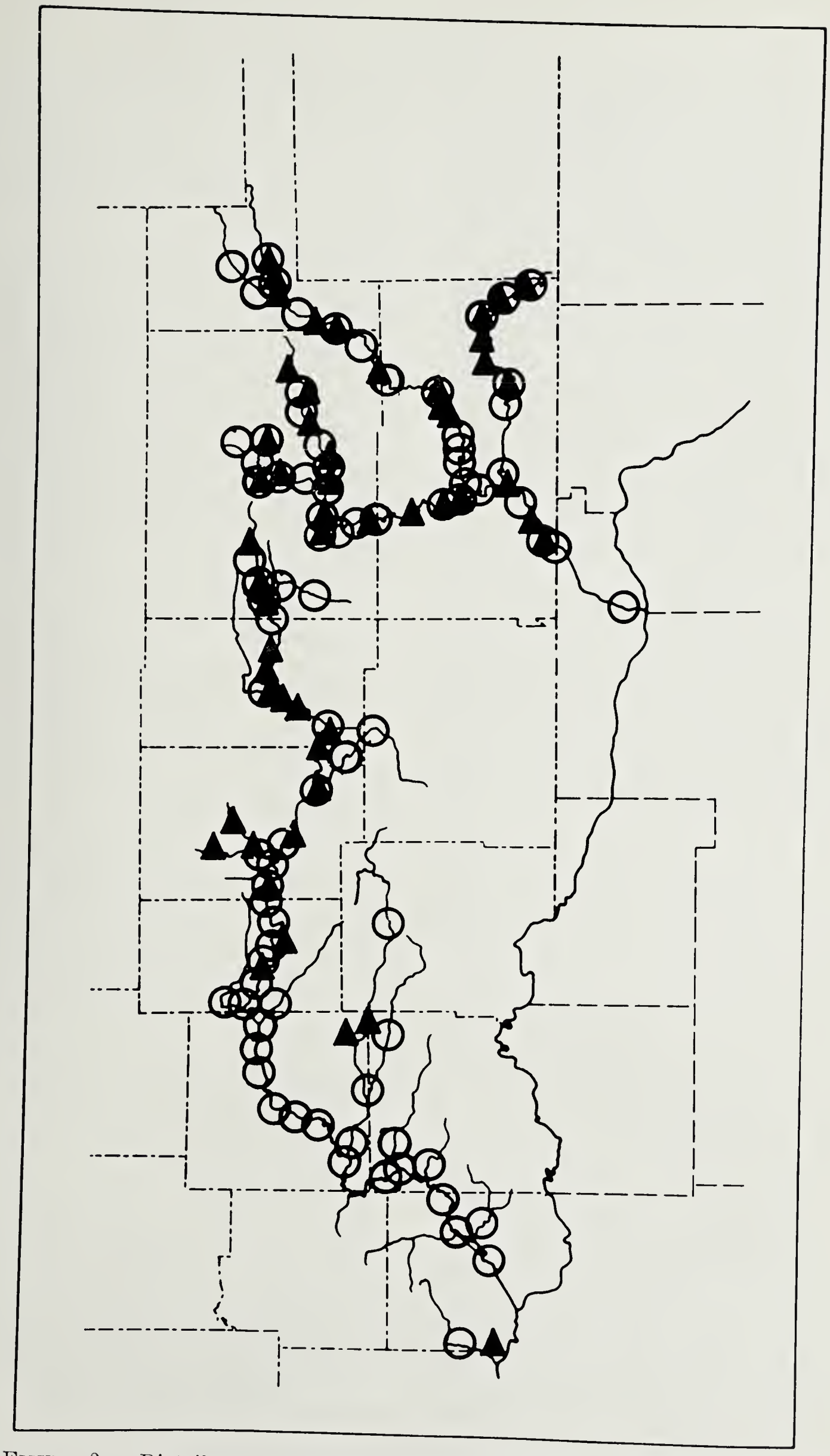


FIGURE 2. — Distribution of collections made between 1882 and 1901 (solid triangles) and those made between 1956 and 1966 (open circles).

made over 100 collections at 86 sites in the two basins (Fig. 2). Although a few collections and published observations are available for the period between 1901 and 1956, most of the data presented herein consist of a comparison of present distributional patterns with those of the pre-1901 investigation (Forbes & Richardson 1920, atlas of maps).

Nearly 100 species of fishes have been reported from the Vermilion and Embarras River basins. Because of the more intensive sampling possible at the present time, the majority of the species of fishes are now known from considerably more sites than they were prior to 1901. However, one species, the blacknose shiner, has evidently been extirpated from the area, and another 20 species show irrefutable evidence of decimation, particularly in the North Fork, West Branch of the Salt Fork, lower Vermilion River, and in the Embarras River above the city of Charleston. These 21 species and the probable reasons for their decimation in the two drainages are the basis of this paper.

By using judiciously our knowledge of the present habits and habitat of each species involved, we can ascertain the factor or factors responsible for the decimation of that species and then proceed to analyze the modifications of stream and watershed that have produced these factors.

Extirpated and Decimated Species

The extirpated blacknose shiner, if the species were correctly identified at that time as it appears to have been, was reported from tributaries of the North Fork, West Branch of the Salt Fork, and the middle Embarras River. The species, probably widespread in the lakes, prairie sloughs, and clear connecting streams, evidently disappeared with the initial drainage of the wet prairie in eastern Illinois.

Another species, the Ohio lamprey, was reported from two localities in the Embarras River by Hubbs & Trautman (1937:87) and does not now occur in the drainage or even in Illinois (Starrett, Harth, & Smith 1960). However, it has been omitted from the present analysis because the Embarras River records may have been based on misidentified specimens of the allied chestnut lamprey.

Nine species no longer occur in parts of the Vermilion and Embarras River basins where they were taken prior to 1901. These species, characteristic of fast riffles or clear flowing water in shallow pools over a sand or gravel substrate, are almost certainly decimated because riffle and hard-bottomed-pool habitats have been destroyed in many areas:

Hog sucker
Stoneroller
Silverjaw minnow
Suckermouth minnow
Brindled madtom
Greenside darter
Fantail darter
Eastern sand darter
Blackside darter

Five other species, characteristic of clear pools with luxuriant vegetation, are less widely distributed than formerly, probably because of the silt-laden water in pools of today and the disappearance of aquatic vegetation:

Spotted sucker
Bigeye chub
Bigeye shiner
Brook silverside
Black crappie

Three species, characteristic of large rivers, evidently retreated downstream and did not return as the middle and upper reaches of the Vermilion and Embarras rivers diminished in size, probably when periods of drought first began to re-

duce seriously the flow of these streams:

Silver chub
Bullhead minnow
Mud darter

At least three species, characteristic of soft-bottomed pools and tolerant of silt and some pollution, show rather clear-cut evidence of decimation in both systems and throughout east-central Illinois. Their decline in an area where their preferred habitat seems to be increasing is not readily explained but nevertheless is real, for two of them (black bullhead, orangespotted sunfish) were cited by Larimore & Smith (1963:332) as decimated in Champaign County during the same period:

Black bullhead
Tadpole madtom
Orangespotted sunfish

Principal causes for decimation.—If our inferences regarding the reasons for decimation of the first 17 of the 20 species cited are correct, we must assume that the principal factors affecting fishes of the Vermilion and Embarras River systems are (1) alteration of the physical habitat, e.g. rate of flow, bottom type, (2) an increase in amount of suspended silt and subsequent loss of aquatic vegetation, and (3) a reduction in stream size.

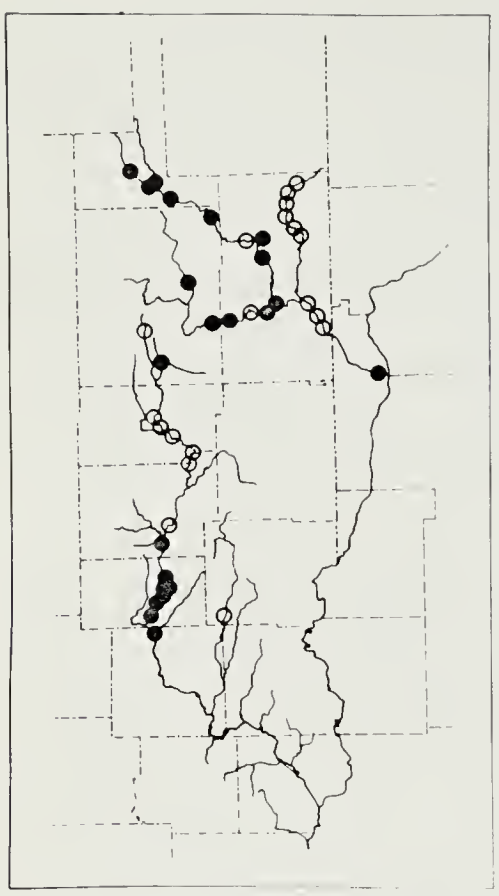
While pollution has undoubtedly contributed, its effects are often much less dramatic and even difficult to discern. When severe pollution abates, fishes are quickly recruited from tributary streams and downstream habitats and soon return to their original habitats.

A notable illustration of decimation that can be attributed to chronic pollution is in the West Branch of the Salt Fork, where Forbes & Richardson (1920, atlas of maps) collected at least 16 species that do not presently occur in the stream. Chronic pollution may also have contributed to the apparent decimation of a few species in the Vermilion River below the city of Danville.

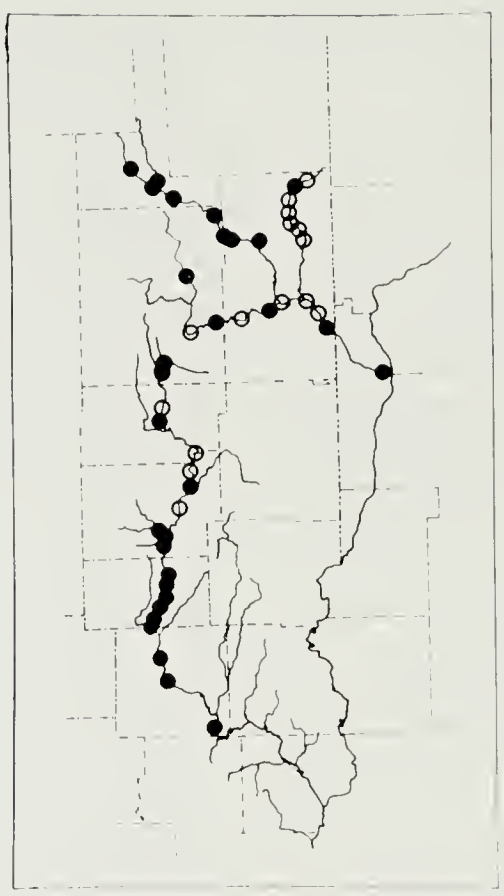
Man-made modifications that have altered the physical habitat in this region, e. g. rate of flow, bottom type, include dams and artificial impoundments on the North Fork and the Embarras River, dredging in the upper reaches of the Vermilion River, canalizing in the lower Embarras River, and deposition of sludge from the Danville sewage plant in the lower Vermilion River.

An artificial impoundment drowns riffles and reduces flow, resulting in siltation which eventually produces a mud bottom, murky water, and a loss of desirable types of aquatic vegetation. While creating a recreational lake of several acres near the dam site, the impoundment alters the stream for many miles upstream from the recreation area. Fishes characteristic of flowing water and firm bottoms are replaced by those characteristic of oxbows and deep, soft-bottomed pools, not only in the reservoir but for many miles above it.

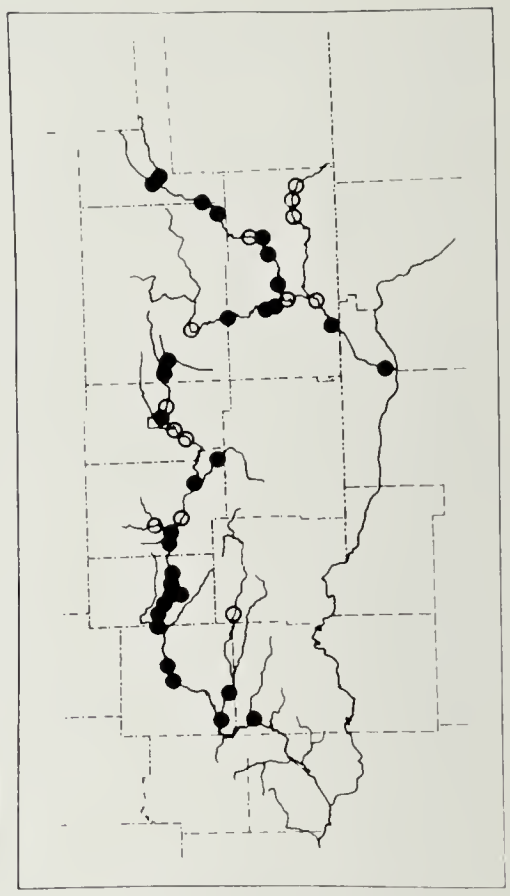
Rather dramatic illustrations of the effects of the impoundment, particularly in the North Fork and Embarras River above Charleston, are provided by the present ranges of the greenside darter (Fig. 3), northern hog sucker (Fig. 4), brindled madtom (Fig. 5), blackside darter



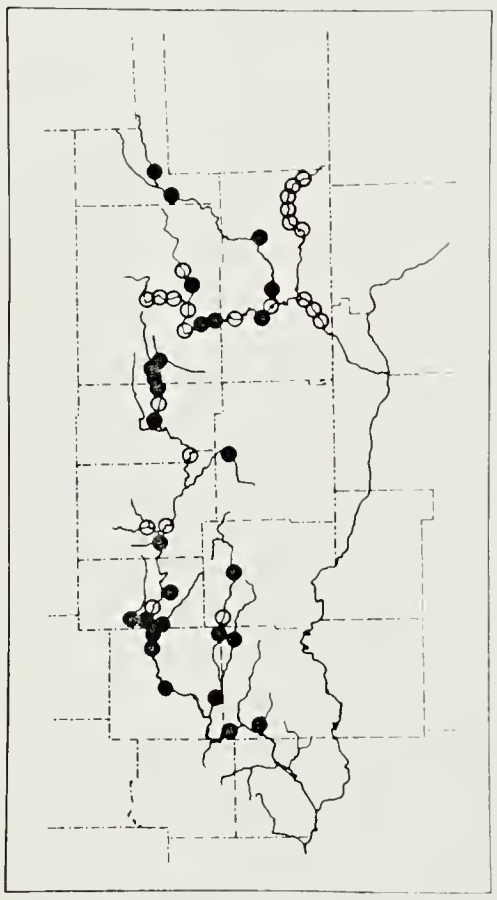
GREENSIDE DARTER



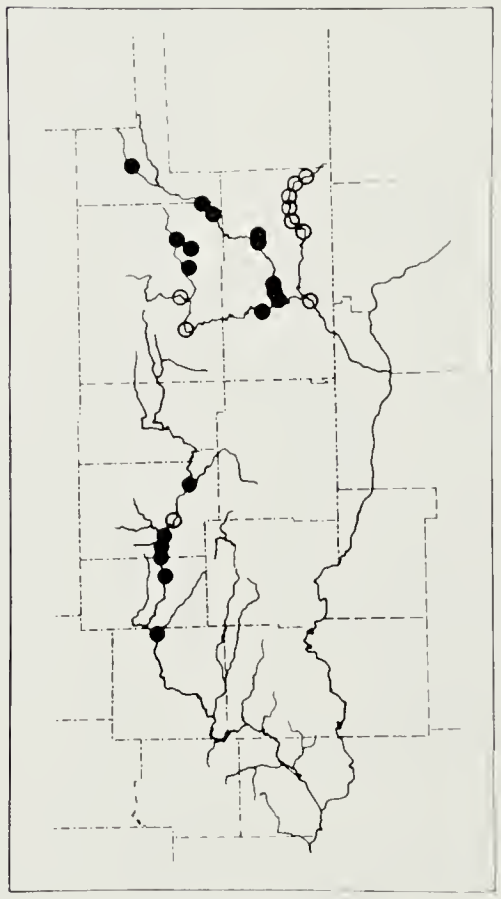
NORTHERN HOG SUCKER



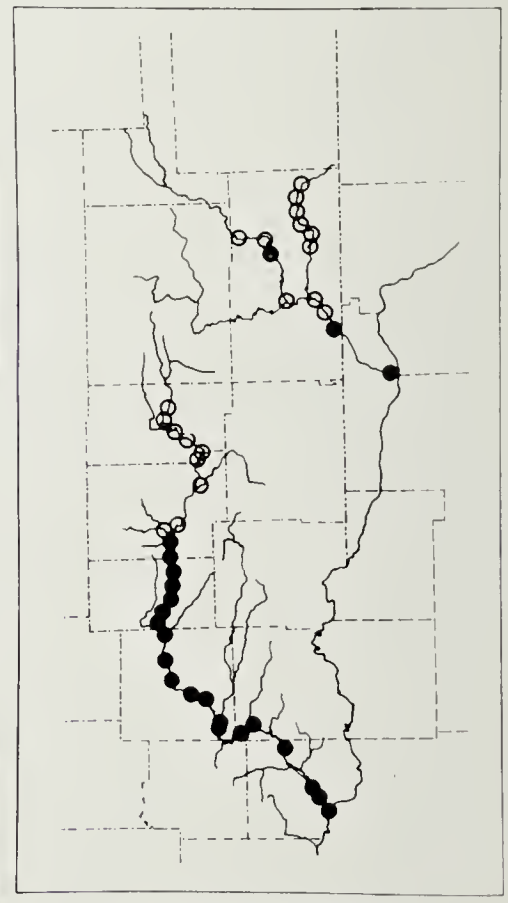
BRINDLED MADTOM



BLACKSIDE DARTER



FANTAIL DARTER



BULLHEAD MINNOW

FIGURES 3-8. — Former distribution (open circles) and present distribution (solid symbols) of six species of fishes decimated in the Vermilion and Embarras River basins as a result of dams on the lower North Fork and Middle Embarras River.

(Fig. 6), and fantail darter (Fig. 7). Loss of fast riffles and silt deposition in pools that once had firm bottoms resulted in their disappearance from areas where they were generally distributed prior to the erection of dams.

Dams and impoundments further serve as insurmountable barriers to upstream dispersal of large-river and channel species, which quickly disappear above the dams and, unlike small-stream species, cannot be recruited from lesser tributaries. An example is the bullhead minnow (Fig. 8), which formerly was generally distributed in the North Fork and Embarras River and known from a few localities on the Middle Fork. The species is presently unable to reoccupy the North Fork and Embarras River, even when these streams are temporarily large rivers, because of the dams. In the Embarras River, the bullhead minnow occurs in abundance virtually up to the Lake Charleston dam.

Although precise measurements of water turbidity and observations on the amount of aquatic vegetation present are not available for comparison, there is no doubt that the quantity of silt has increased in the streams and that both submerged and marginal aquatic vegetation have decreased in the Vermilion and Embarras River systems. Fishes that once occupied clear, firm-bottomed and well-vegetated pools are now sporadic in occurrence in the two watersheds and throughout Illinois. Noteworthy examples are the bigeye chub (Fig. 9), bigeye shiner (Fig 10), and spotted sucker (Fig. 11).

Dredging changes the stream from one consisting of alternating riffles with hard bottoms and pools

FIGURES 9-11. — Former distribution (open circles) and present distribution (solid symbols) of three species of fishes decimated in the Vermilion and Embarras River basins as a result of silt-laden water and loss of vegetation in formerly clear, hard-bottomed pools.

with hard or soft bottoms to a relatively straight, diked ditch with rather uniform flow, depth, and bottom type, usually mud or sand depending upon the prevailing soil texture in the area. Vegetation and brush are removed, and all existing habitats temporarily destroyed. With a loss in the number of available habitats, the number of species in the stream is reduced. When the stream reverts to something of its original condition, another dredging is undertaken.

Canalization has much the same effects, except that habitat disturbance is more severe. Small ditches tend to be moderately fast flowing; large ones, sluggish and soon littered with logs and other debris. Its effects upon the extensively rechanneled lower Embarras River cannot be assessed since no early collections of fishes are available for study.

Sludge deposition in the Vermilion River below the Danville sewage treatment plant obliterates the sand and gravel in riffles and pools. The number of species present is reduced as less tolerant fishes are eliminated and spawning sites are destroyed by the layer of sludge. Because of the enrichment of the water, the number of individual fish is high, but many of them have eroded fins and other pollution-induced anomalies.

Reduction in stream size results from accelerated drainage of the watershed and the average lowering of the water table. Large, once stable streams become small, less stable streams, which are more profoundly affected by drought. When tributaries become dry, small-stream fishes perish or retreat to the parent river; when the tributaries again have water in them, fishes from nearby habitats quickly repopulate tributaries. However, when the large stream diminishes and large-river species die or move downstream, the short period of time between low-water stages and the great distance fishes must reascend prevent their reoccupying their original haunts. As previously illustrated by the range of the bullhead minnow (Fig. 8), a dam or other obstruction blocks upstream dispersal, even when the river has temporarily regained its large size.

Species possibly benefiting from the changing environments.—It is axiomatic that typically lacustrine fishes may also inhabit streams, but a substantially smaller percentage of typically stream-inhabiting fishes occur in lakes. With the extirpation of the blacknose shiner, none of the native fishes of the Vermilion and Embarras River systems is truly a large-lake species. The construction of large reservoirs thus creates large-lake niches that can be occupied either by introduced lake species or native species actually characteristic of oxbows, backwater areas, or quiet pools of streams.

While the conditions responsible for the decimation of most of the 20 species previously cited can be enumerated, it is reasonable to assume that these same conditions should have benefited, to some extent, species characteristic of oxbows and quiet, murky, soft-bottomed pools. Among these are the following species, most of which are now common in one or both systems:

Gizzard shad

Creek chubsucker
Carp
Golden shiner
Redfin shiner
Fathead minnow
Creek chub
Black bullhead
Yellow bullhead
Tadpole madtom
Pirate perch
Blackstripe topminnow
Mosquitofish
Warmouth
Green sunfish
Orangespotted sunfish
Bluegill
Largemouth bass
White crappie
Bluntnose darter
Slough darter

Although the assumption is reasonable, no data are presently available to indicate that any of these species are more abundant or more generally distributed than formerly, and three of them (black bullhead, tadpole madtom, and orangespotted sunfish) have, in fact, been cited on a preceding page as species decimated in the two basins.

Endangered Populations of Fishes

At this writing (March 1967), the Corps of Army Engineers has scheduled, and the Congress has authorized, the construction of a dam on the Embarras River at the Coles-Cumberland County line to create the proposed Lincoln Reservoir, an impoundment to have a seasonal pool covering 6,760 acres and to extend almost 30 straight-line miles above the dam. The reservoir, one of several projects planned for the Wabash River valley, is allegedly designed as a flood-control lake and recreation site; it would also provide a water supply for the city of Charleston, a community that has had water shortages on several occasions in recent years.

The Illinois Division of Waterways has recommended, but the State Legislature has not yet authorized, the construction of a dam on the Middle Fork near Kickapoo State Park. This proposed reservoir, designed to assure a water supply for the city of Danville and to provide additional recreational space, would inundate an unknown number of acres and extend about 12 miles above the dam.

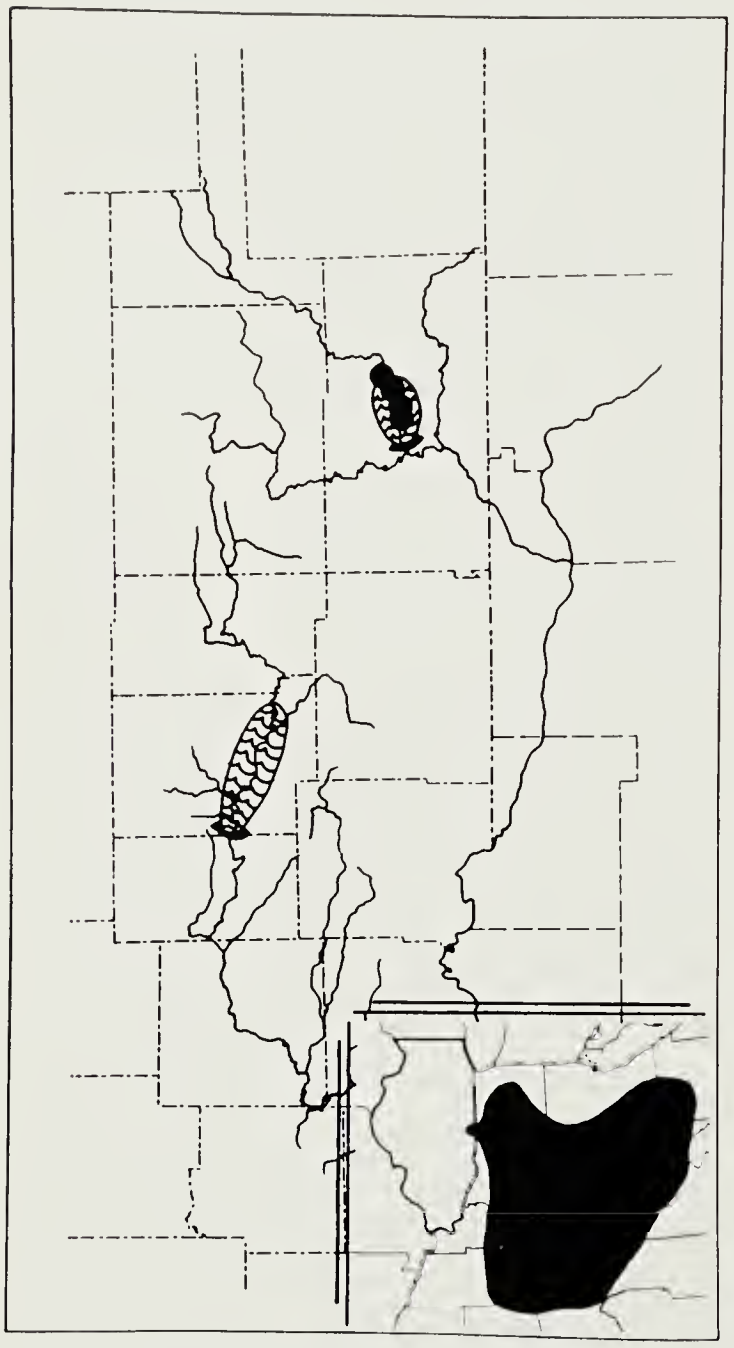
The Illinois Department of Conservation has scheduled, and has received some appropriations from the State Legislature, the construction of dams on tributaries of the Salt Fork and Embarras River to create a small conservation lake in Champaign County and another in Douglas County.

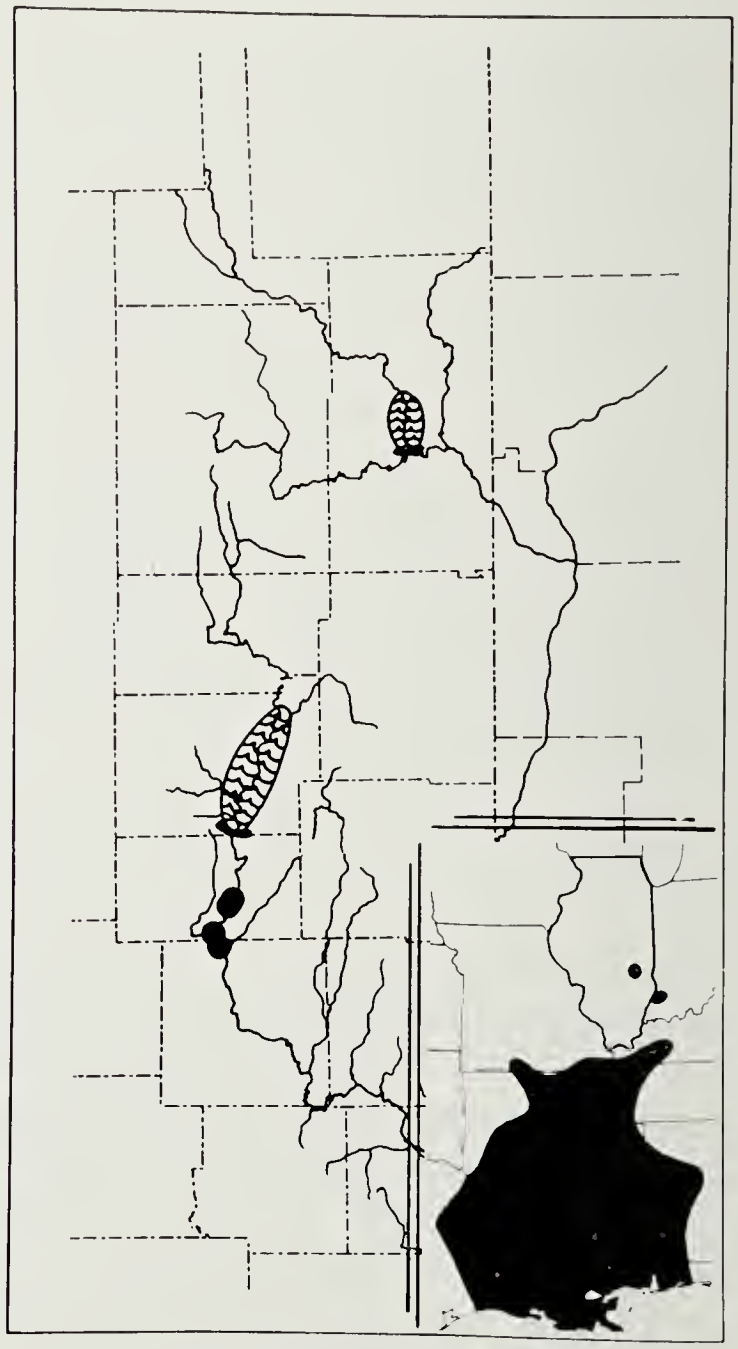
The Illinois State Water Survey, although not recommending any specific dams, has conducted an independent survey of potential surface water reservoirs based on feasibility. This agency noted that a good dam site was available on the Embarras River near that of the proposed Lincoln Reservoir dam but described the project as, "probably not feasible geologically until further investigation proves that leakage can be controlled" (Dawes & Terstriep, 1966a:34). The Water Survey identified almost 50 other sites, mostly on tributaries of the Embarras River, as being feasible for construction of small reservoirs

but did not include the Middle Fork site or those selected by the Department of Conservation (Dawes & Terstriep 1966a; 1966b).

Aside from further, rather extensive decimation of some stream fishes through destruction of their habitats, the Lincoln Reservoir would probably not greatly affect any Illinois species of fish, and there is no evidence that construction of small reservoirs on tributaries of the Vermilion and Embarras rivers would have any appreciable effect upon the general distribution of Illinois fishes. However, the Middle Fork Reservoir, should it become a reality, would surely eliminate in Illinois one species of darter, and future reservoirs at other sites on the Vermilion and Embarras rivers could jeopardize Illinois populations of some of the following fishes.

Bluebreast darter. — This species occurs in an approximately 12-mile stretch of the Middle Fork (Smith 1965:10), the entire length of which would be affected by a dam at Kick-





FIGURES 12-13. — Illinois distribution of the bluebreast and harlequin darters. Shading depicts areas that would be affected by contemplated reservoirs. Inset maps indicate total ranges of the species.

apoo State Park (Fig. 12). The bluebreast darter inhabits extremely fast riffles containing boulders as well as gravel and rubble, and it spawns near boulders or large rocks at the heads of these riffles. Although occurring in adjacent Indiana, the species has not been found in Illinois anywhere except in the lower Middle Fork.

Harlequin darter.—Although not currently threatened by a proposed reservoir, this species has a similarly restricted range in Illinois (Fig. 13). It is known to occur in roughly a 10-mile stretch of the middle Embarras River (Smith 1965:10), where it is found sparingly in deep, sand-bottomed riffles containing submerged logs or stumps. If the dam for the proposed Lincoln Reservoir were 15 miles farther downstream or if a dam should be erected in that vicinity in the future, the species would almost certainly be eliminated from Illinois. Unlike the bluebreast darter, this species has been recorded from only one locality in Indiana (Jordan 1890) and never collected in that state again. The Illinois population is thus the only northern one extant for this little-known darter.

Eastern sand darter.—This eastern species reaches its westernmost limit of range in the Wabash-Ohio River basin of eastern Illinois. It is virtually restricted to the Embarras River and Middle Fork, for 12 of the 13 known Illinois records are from these two rivers (Fig. 14). Intolerant of silt and habitat disturbance, the species inhabits clear riffles and shallow pools over bottoms of clean sand.

Dusky darter.—This wide-ranging species is similar in its Illinois distribution to the eastern sand darter, inasmuch as 23 of the 26 known Illinois records are from the Vermilion and Embarras river basins (Fig. 15). It inhabits clear, fast gravelly riffles and disappears when rate of flow and bottom type are altered in the stream.

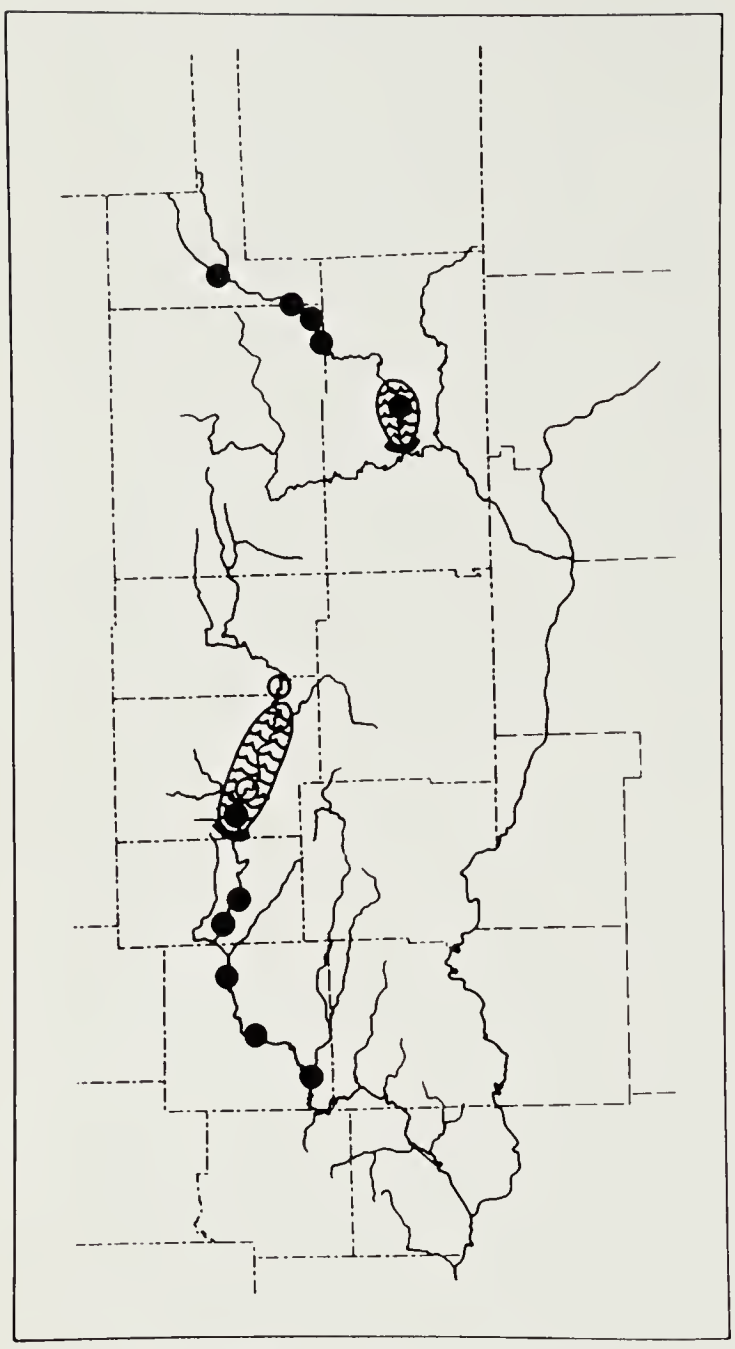
A few species of shiners and madtoms and the spotted bass, although not restricted to the Vermilion and Embarras river systems, also reach their greatest abundance, and are best known in Illinois, in these two basins.

The number of endangered species in Illinois waters is quite small and, if contemplated reservoirs materialize, only one of them is destined for extirpation. However, evidence presented in an earlier section of this paper clearly indicates that extensive decimation of populations of fishes characteristic of riffles and clear, hard-bottomed pools would be inevitable in both the Middle Fork and Embarras River and that other changes in the fish fauna of the two systems are likely to follow construction of new dams.

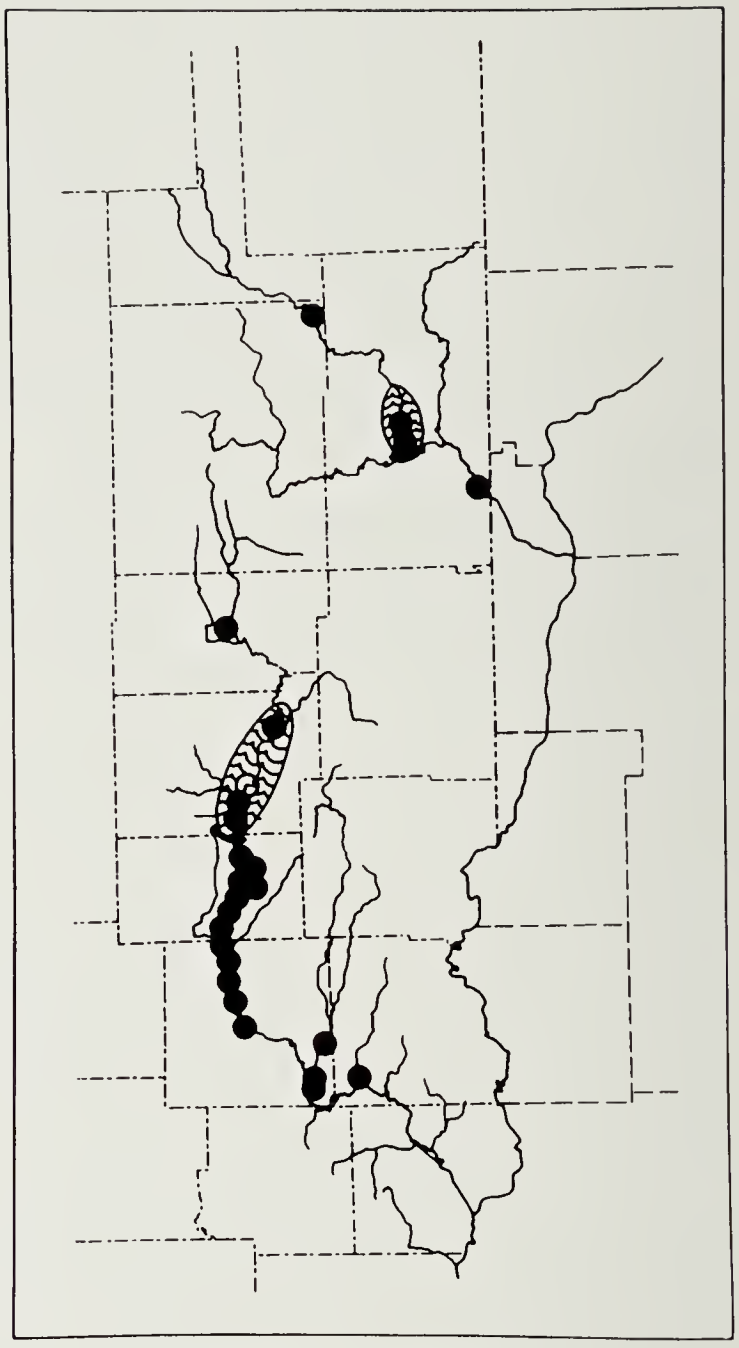
Pollution, including siltation, and other modifications of the streams and watersheds, will continue to intensify ecological hazards for fishes with restricted distribution, but currently alteration of the physical habitats through construction of artificial reservoirs on main streams, the one activity that could be circumvented, is clearly the greatest threat to them.

Acknowledgments

I have profited from discussions of material covered in this paper with Survey



EASTERN SAND DARTER



DUSKY DARTER

FIGURES 14-15. — The distribution in the Vermilion and Embarras River basins of the eastern sand and dusky darters. Shading depicts areas that would be affected by contemplated reservoirs.

colleagues Drs. George W. Bennett, R. Weldon Larimore, and William C. Childers; Chief Fishery Biologist A. C. Lopinot, Illinois Department of Conservation Division of Fisheries; and Dr. Leonard Durham, Eastern Illinois University. Mr. Julius H. Dawes of the Illinois State Water Survey furnished information on both Lake Charleston and Lake Vermilion, and Dr. Edward C. Raney, Cornell University, supplied distributional data on the range of the harlequin darter from a thesis by his former student Chu-fa Tsai.

The maps accompanying this article were drafted by Richard M. Sheets, Survey Technical Illustrator, and prints were made available to me by Wilmer D. Zehr, Survey Photographer. Drs. H. H. Ross and George Sprugel, Jr., read the manuscript and offered helpful suggessions.

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Manuscript received April 13, 1967

Appendix

Scientific Names of Species of Fishes Cited in Text

Ohio lamprey, *Ichthyomyzon bdellium* (Jordan)
Chestnut lamprey, *Ichthyomyzon castaneus* Girard
Gizzard shad, *Dorosoma cepedianum* (Lesueur)
Stoneroller, *Campostoma anomalum* (Rafinesque)
Carp, *Cyprinus carpio* Linnaeus
Silverjaw minnow, *Ericymba buccata* Cope
Bigeye chub, *Hybopsis amblops* (Rafinesque)
Silver chub, *Hybopsis storeriana* (Kirtland)
Golden shiner, *Notemigonus crysoleucas* (Mitchill)
Bigeye shiner, *Notropis boops* Gilbert
Blacknose shiner, *Notropis heterolepis* Eigenmann & Eigenmann
Redfin shiner, *Notropis umbratilis* (Girard)
Suckermouth minnow, *Phenacobius mirabilis* (Girard)
Fathead minnow, *Pimephales promelas* Rafinesque
Bullhead minnow, *Pimephales vigilax* (Baird & Girard)
Creek chub, *Semotilus atromaculatus* (Mitchill)
Creek chubsucker, *Erimyzon oblongus* (Mitchill)
Northern hog sucker, *Hypentelium nigricans* (Lesueur)
Spotted sucker, *Minytrema melanops* (Rafinesque)
Black bullhead, *Ictalurus melas* (Rafinesque)
Yellow bullhead, *Ictalurus natalis* (Lesueur)
Tadpole madtom, *Noturus gyrinus* (Mitchill)
Brindled madtom, *Noturus miurus* Jordan
Blackstripe topminnow, *Fundulus notatus* (Rafinesque)
Mosquitofish, *Gambusia affinis* (Baird & Girard)
Brook silverside, *Labidesthes sicculus* (Cope)
Pirate perch, *Aphredoderus sayanus* (Gilliams)
Warmouth, *Chaenobryttus gulosus* (Cuvier)
Green sunfish, *Lepomis cyanellus* Rafinesque
Orangespotted sunfish, *Lepomis humilis* (Girard)
Bluegill, *Lepomis macrochirus* Rafinesque
Spotted bass, *Micropterus punctulatus* (Rafinesque)
Largemouth bass, *Micropterus salmoides* (Lacépède)
White crappie, *Pomoxis annularis* Rafinesque
Black crappie, *Pomoxis nigromaculatus* (Lesueur)
Eastern sand darter, *Ammocrypta pellucida* (Baird)
Mud darter, *Etheostoma asprigene* (Forbes)
Greenside darter, *Etheostoma blennioides* Rafinesque
Bluebreast darter, *Etheostoma camurum* (Cope)
Bluntnose darter, *Etheostoma chlorosomum* (Hay)
Fantail darter, *Etheostoma flabellare* Rafinesque
Slough darter, *Etheostoma gracile* (Girard)
Harlequin darter, *Etheostoma histrio* Jordan & Gilbert
Blackside darter, *Percina maculata* (Girard)
Dusky darter, *Percina sciera* (Swain)

INTERSECTING BELTS OF POST-TECTONIC "ALKALINE" INTRUSIONS IN NEW ENGLAND

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ABSTRACT.—Magmatic central complexes and plutons of "alkaline" nature, emplaced post-tectonically, largely by stoping, are widespread in New England and southern Quebec. They are concentrated within two principal belts. A north-northwest belt is composed largely of intrusions of the White Mountain magma series in New Hampshire and the Monteregian Hills of Quebec. The northeast belt is represented by intrusions along the eastern half of the Maine coast. Petrographic, structural, and geochemical evidence indicate that the northeast belt of plutons, together with the Bays-of-Maine igneous complex, extends southwestward into Connecticut and is transected in eastern Massachusetts by the north-northwest intrusion belt. Such a relation, if correct, may account for the two rather distinct sets of ages (180 and 320 m.y.) obtained for the "alkaline" intrusions in central and southern New England.

Plutonic bodies ranging in age probably from Ordovician to Cretaceous and in composition from gabbro to syenite and granite are abundant and widely distributed over New England and southern Quebec. For convenience they are divided into three groups: pretectonic, syntectonic, and post-tectonic. Although we will be concerned in this paper with bodies of the last group, it is recognized that all gradations occur between the syntectonic and post-tectonic types. To define our group here more precisely, we will consider those bodies which on the whole show no apparent effects of orogeny, are mildly alkaline in character, and generally constitute magmatic central complexes emplaced in the observable portion of the crust by ring-fracture stoping. For simplicity these "alkaline" intrusions are considered to belong principally to two geographic belts, one trending north-northwest and the other northeast (Figure 1).

THE NORTHEAST INTRUSION BELT

The northeast belt is located along the eastern half of the Maine Coast and coincides, at least in part, with a gigantic assemblage of mafic and felsic rocks known as the Bays-of-Maine igneous complex (Chapman, 1962). The complex, at least 175 miles long, extends well into New Brunswick in one direction and across Penobscot Bay, in south-central Maine, in the other. It was emplaced largely within and beneath a thick pile of volcanic and associated sedimentary rocks (Silurian and Devonian) in the late stage of Acadian orogeny. This may account for its trend parallel to tectonic strike. An early phase of the complex is represented by gabbroic and dioritic rocks with both visible and cryptic layering. More or less on top of the mafic phase and beneath a roof of volcanic rocks is an extensive sheet or capping of the younger, gianitic-granophyric phase.

Cutting through both phases of the complex are at least 14 granitic plu-

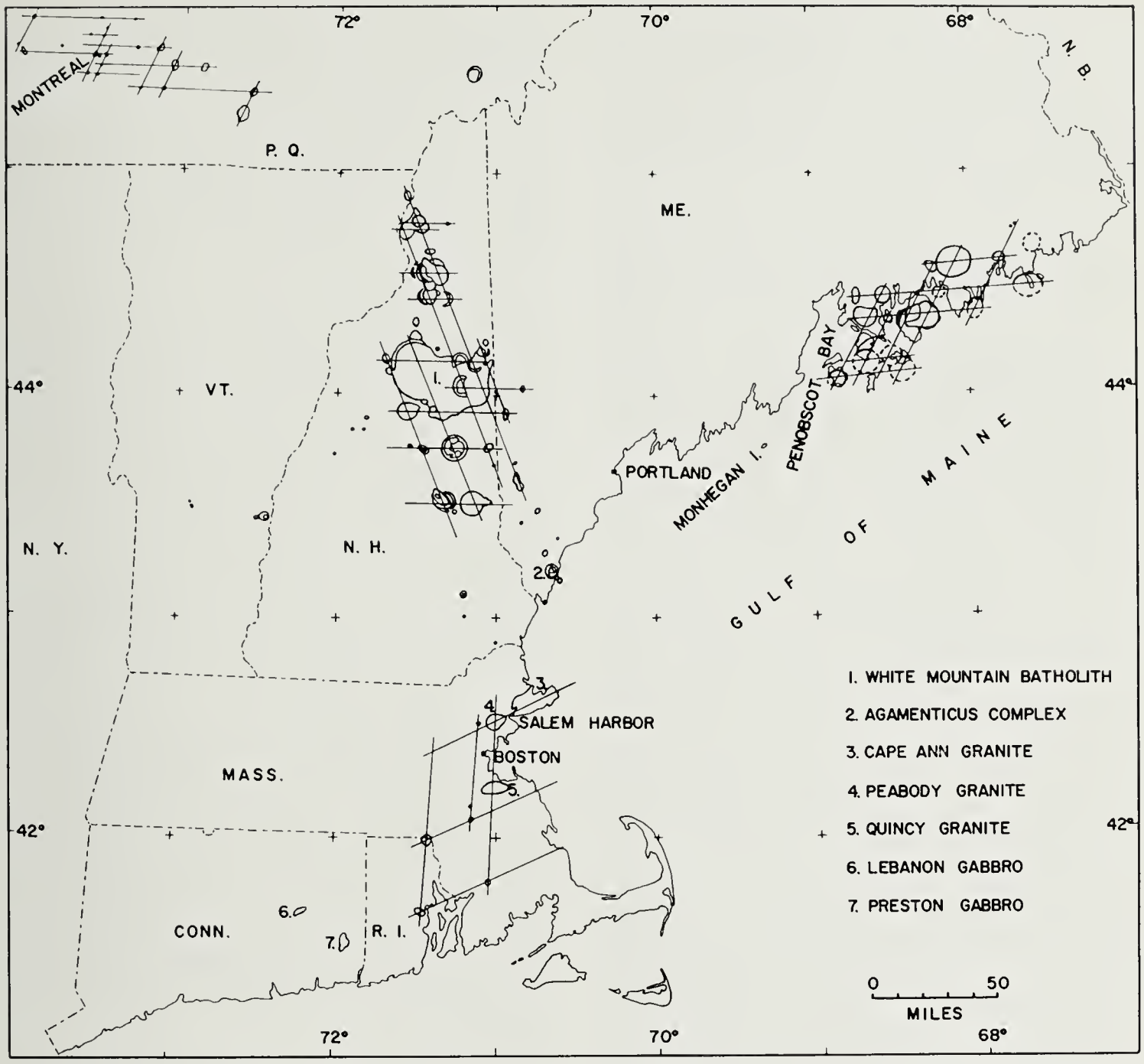

FIGURE 1. Distribution of post-tectonic "alkaline" intrusions in New England and southern Quebec.

tons, which in many respects resemble the granitic bodies of the north- northwest belt. They form a belt at least 25 miles wide and 80 miles long. These dimensions are limited by the Gulf of Maine, which undoubtedly covers numerous other plutons. Previously (Chapman, 1962) these granites were referred to as the "younger granites" and were separated from the Bays-of-Maine igneous complex. As study in the area progresses if may appear more logical to group the "younger granites" with the great complex as a third and later phase.

For the most part the plutons are roughly circular, 5-10 miles in diameter, and stock-like in form. A few ring-dikes are known. Granite and quartz monzonite are virtually the only rock types observed. Volcanic rocks genetically related to the granites have not yet been recognized. The plutons are disposed in a reticulate pattern in which two directions of linear arrangement (N30°E and N85°E) intersect at 55° (Chapman, 1967).

The mafic phase of the Bays-of-Maine igneous complex was emplaced in stratified rocks of Silurian and Devonian age, and it shows some effects of Acadian orogeny. The younger granitic-granophyric phase is overlain unconformably by the Upper Devonian, Perry Formation in eastern Maine (Bastin and Williams, 1914). Here, Faul et al (1963) date this phase as 404 m.y. It appears then that the Bays-of-Maine igneous complex in eastern Maine is roughly of Early Devonian age.

To the southwest toward Penobscot Bay the "younger granites" are abun-

dant and appear to cut clearly through the great complex. An upper geologic age limit for these intrustions has not been established. Several age determinations (Faul et al, 1963), however, indicate a wide range for these bodies from 354-399 m.y. (Early to Late Devonian).

The North - northwest Intrusion Belt

The north - northwest belt extends across New Hampshire and is constituted by intrusions of the White Mountain magma series (Billings, 1956). It is roughly 40 miles wide and 150 miles long. The igneous rocks are principally plutonic and include mostly granite with subordinate syenite and quartz syenite and minor amounts of gabbroic and intermediate types. The intrusions of this series exhibit a striking bilaterally symmetrical distribution along the belt relative to size, shape and composition; and several features should be noted.

1. In the central part of the belt are large contiguous masses of plutonic rocks forming the White Mountain batholith (Billings, 1956). These plutons are stock-like in outline, but a few poorly formed ring-dikes are recognized. Compositionally the rocks are predominantly granite; only a small percentage is quartz syenite and syenite. Mafic types are essentially absent.

2. Both to the north-northeast and south-southwest, away from the batholith, are smaller but discretely disposed intrusive complexes. Here we find the large, well-formed ring-dikes of quartz syenite, syenite, and less commonly granite as well as numerous various-sized stocks mostly of granite. Mafic masses are important only locally.

3. Beyond the large discrete complexes are smaller complexes and plutons. In southern New Hampshire and southwestern Maine the series appears to be represented by small stocks, plug-like bodies, and perhaps funnel-shaped masses (Hussey, 1962). Excepting the Agamenticus complex here, the rocks are dominantly gabbroic and intermediate in composition. Toward the other end of the belt, in northern New Hampshire and Vermont, small plug-like and funnel-like masses do not appear.

4. As was true of the Maine coastal plutons, the intrusions of the White Mountain magma series are disposed along two well-defined lines (22°W and E-W), which intersect (at approximately 68°) to form a reticulate pattern (Chapman, 1963).

5. Changes along the intrusion belt appear more striking and systematic than changes across the belt. In a very general way, however, intrusive bodies are smaller toward either flank of the belt.

6. A central zone of intense metamorphism and plutonism (Late Devonian) in New Hampshire gives way northwestward and southeastward to successively less intense zones (Billings, 1956). This bilaterally symmetrical pattern, furthermore, is roughly coincidental with the bilaterally symmetrical pattern of the White Mountain magma series already described.

7. The northeasterly trending regional structure and metamorphic zones in New Hampshire are transected by the north-northwest intrusion belt at about 50°.

8. Lyons and Faul (in press) give the age for the White Mountain magma series as 180 m.y. (Early Jurassic).

From these observations it now appears that the post-tectonic "alkaline" instrusions of New Hampshire and neighboring parts of Maine and Vermont constitute a distinct and symmetrically disposed group set apart spatially, temporally, petrographically, and structurally from other plutons in New England.

To the extreme northwest the intrusion belt appears to pass into the belt of Monteregian Hills intrusions in southern Quebec (Dresser, 1906). These northern intrusions resemble in some respects those of southeastern New Hampshire and southwestern Maine for they also are small stock-like and plug-like bodies (some volcanic necks) composed predominantly of mafic and intermediate rocks. Small diatremes are abundant and some ultramafic rocks are represented. Northwestward, from the international boundary to Montreal (100 miles), the intrusions generally become smaller and more mafic. In a general way this group of small bodies fits the symmetrical pattern already described for the White Mountains magma series, and it might be considered the counterpart of the group in southeastern New Hampshire and southwestern Maine.

Three important differences, however, are to be noted. (1) Certain rocks of

the Monteregian Hills have been dated (Fairbairn et al, 1962) as approximately 120 m.y. (Early Cretaceous). (2) The rocks are distinctly more alkalic than the White Mountain magma series as a whole; and in the more southeastern bodies, foidal syenites are moderately abundant. To the northwest essexite becomes increasingly more dominant. (3) The shape and disposition of bodies suggests a tectonic pattern with lattice lines trending N25°E and E-W. A northwesterly trend, however, does not appear as prominent or consistent. These differences, though very definite, are not in the least detrimental to the idea that the Monteregian Hills intrusions are very closely linked genetically to those of the White Mountain magma series. Both groups of intrusions may be considered as belonging to a single belt.

Extending southeast from eastern Massachusetts for roughly 900 miles is the colinear belt of Calvin seamounts, considered of volcanic origin and Late Cretaceous age (Northrop et al, 1962). These features may represent a later and more southeasterly extension of the north-northwest intrusion belt.

Returning to the main belt we find the bilaterally symmetrical distribution of intrusions (relative to size, shape, and composition) on either side of the White Mountain batholith appears to break down in eastern Massachusetts where large stock-like bodies of "alkaline" granite appear again. This might suggest that the "alkaline" plutons of Massachusetts and Rhode Island do not belong strictly to the north-northwest belt.

The age of these "alkaline" rocks is of particular importance in this regard. Emerson (1917, p. 188) demonstrated the pre-Pennsylvanian age for the Quincy Granite (Near Boston); and Williams and Billings (1938, p. 1025) assuming the New England "alkaline" rocks to be contemporaneous, tentatively concluded a pre-Pennsylvanian age for the White Mountain magma series. Since then there has been a tendency to think of all "alkaline" rocks in New England as correlatives of the White Mountain magma series. More recent studies, summarized by Lyons et al (1957) and Toulmin (1961), indicate an age of 260-270 m.y. for the "alkaline" rocks in eastern Massachusetts as compared with 180 m.y. for the White Mountain magma series. Lyons and Faul (in press) now give the geochemical age of the Massachusetts rocks as 320 ± 20 m.y.

Intersection of the Intrusion Belts

If we assume that the somewhat older northeast belt of the Bays-of-Maine igneous complex and associated "younger granites" extends southwestward across eastern Massachusetts and into Connecticut, then the apparent, great discrepancy in rock ages indicated above may be reconciled.

The writer has already suggested (Chapman, 1962, p. 887) that the Bays-of-Maine igneous complex, which extends from central New Brunswick to Penobscot Bay in Maine (175 miles), may disappear ". . . beneath the Gulf of Maine only to emerge again in eastern Massachusetts, 125 miles beyond." The gabbroic rocks of Monhegan Island (Lord, 1900) may represent a portion of the mafic phase; and therefore, the great complex may be definitely extended 25 miles nearer the Massachusetts coast. The writer has long suspected that the mafic phase of the great complex may be represented in eastern Massachusetts by such units as the Salem Gabbro-Diorite, Newburyport Quartz Diorite, and gabbro at Nahant (Emerson, 1917; Toulmin, 1964). Perhaps much of the Dedham Granodiorite represents the felsic phase. In eastern Connecticut the gabbros at Preston (Sclar, 1958) and Lebanon (Foy, 1949) may belong to this belt. It should be noted that bodies of mafic plutonic rock are larger and relatively much more common in the northeast belt than in the north-northwest belt in New England. It is suggested further that the coastal strip of positive gravity anomalies in the Boston-Portland area (Joyner, 1963) may be largely an expression of an unexposed portion of the gabbroic phase of the Bays-of-Maine igneous complex. The "alkaline" granites in eastern Massachusetts and Rhode Island, which can not belong to the White Mountain magma series, therefore, may represent the "younger granites" of the northeast belt.

Geologic observations by Toulmin (1961) and geophysical studies by Joyner (1963) suggest the shape and mechanics of intrusion of the Peabody and Cape Ann plutons resemble in certain respects those features of the Maine coastal plutons. The high gravity anom-

aly over the northeastern part of the Cape Ann granite may be due to a huge subsided block of the mafic phase of the Bays-of-Maine igneous complex (Salem Gabbro-Diorite). Furthermore, the overlap of the gravity high on the Cape Ann Granite just north of Salem Harbor may be due to a down-tilted portion of the mafic rock here. This inward tilt, which would provide a gently dipping shelf upon which feldspar presumably collected (Toulmin, 1964) to form the syenite here, is reminiscent of the external centripetal dip patterns formed around some of the Maine coastal plutons (Chapman, in press). From Joyner's anomaly map it appears to the present writer that something more than a shelf is indicated by the gravity high. Apparently an extensive portion of the Cape Ann Granite is underlain by the mafic rock. This granite may have consolidated, therefore, from a melt which occupied a nearly completely floored chamber.

Just as we found a difference in the geochemical age (ca 50 m.y.) of igneous activity along the north-northwest belt, from the White Mountain magma series of New Hampshire to the Monteregian Hills in one direction and the Calvin seamounts in the other, so might we expect an age difference in the intrusions along the northeast belt. The "alkaline" rocks of eastern Massachusetts and Rhode Island, therefore, may represent activity along the northeast belt that was somewhat later (ca 40 m.y.) than that in the Maine coastal region. Geochemical data, therefore, are in reasonable agreement with the idea that the northeast belt may be extended southwestward through Massachusetts.

Further evidence may be found in the tectonic pattern of instrusions. The Massachusetts - Rhode Island intrusion centers show fair alignment in N3°E and N65°E directions to form an acute lattice angle of 62°. This value is close to that (55°) determined for the Maine coastal plutons. In both areas, furthermore, the acute bisectrix of the lattice pattern roughly parallels the regional tectonic strike.

Significance of Pluton Distribution

A distinctive worldwide characteristic of post-tectonic plutons and magmatic central complexes appears to be their reticulate arrangement (Chapman, 1963, 1967, and in press). A great many of the younger igneous intrusions of New England and southern Quebec form large groups within which the bodies are arranged in two linear directions that intersect to produce a lattice pattern. Four such groups include the intrusions of the Monteregian Hills, White Mountain magma series, Maine coastal region, and eastern Massachusetts and Rhode Island. These reticulate patterns are considered to be controlled by two intersecting sets of deep - seated fractures, or sufaces of low pressure, that initiated the magmatic activity (Chapman, 1963, 1967).

Although the magmatic central complexes and younger plutons are widely spread over the New England region, they appear to be heavily concentrated within two belts. A northeast belt parallels the tectonic trend in New England; a younger, north-northwest belt transects this trend at roughly 50°. This suggests a still grander scale structural control of deep-seated nature.

Different ages may be assigned to magmatic activity in these two belts, and it appears that within either belt intrusive bodies may range widely in age. Eruption spanned a significantly greater period of time in the northeast belt. It began in the early Paleozoic with extensive volcanism (largely Silurian). During the Late Silurian and Early Devonian and somewhat overlapping Acadian orogeny, the mafic and felsic phases of the Bays-of-Maine igneous complex developed along the Maine coast. The time of for-

mation of this great complex in the Massachusetts area is not clear, but it may have been roughly the same. In Early and Middle Devonian time granitic plutons and central complexes formed in the Maine coastal region; but the development of their counterparts to the southwest, in eastern Massachusetts and Rhode Island, appears not to have taken place until Mississippian time.

In the younger, north-northwest intrusion belt igneous activity appears to have passed in time and space from the White Mountain magma series (Early Jurassic) of New Hampshire to the Monteregian Hills intrusions (Early Cretaceous) in one direction and the Calvin seamounts (Late Cretaceous) in the other.

The pronounced shift in igneous activity from the northeast belt along the Maine coast, which parallels tectonic trend, to the north-northwest belt in New Hampshire, which transects the tectonic strike at approximately 50°, appears to reflect changes in the nature and behavior of the crust and subcrustal material beneath New England and southern Quebec. There appears to be nearly complete continuity in the space-time-environment relationship of igneous activity from at least the tectonically older (roughly Middle Devonian) calc-alkalic plutons, so abundant throughout the New England region, to the younger intrusions of the north-northwest belt. The writer (Chapman, 1967) has indicated this continuity by analogy with the Newer Granites of the Scottish Caledonian belt. The general picture developed there by Read (1961) fits admirably the Devonian counterparts of the Acadian belt in New England.

The coincidence of the symmetrical pattern of metamorphism and plutonism with that of "alkaline" intrusive activity in the north-northwest belt may be considerably more than fortuitous. It may indicate the control of crustal thickness, composition, and physical nature on igneous activity along a major tectonic zone. Presumably the disposition of intrusions in a contiguous fashion in the White Mountain batholith as compared with a discrete arrangement further away is principally an expression of differences in eruptive intensity or "eruptive density". Perhaps the greater abundance and proportion of granite in the batholith is due to a thicker and more sialic, and perhaps even warmer, crustal layer in this part of the belt. Some differences along the belt may be attributed to differences in levels of observation.

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Manuscript received April 29, 1967.

A FLORISTICS STUDY OF BELL SMITH SPRINGS, POPE COUNTY, ILLINOIS

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ABSTRACT.—A detailed botanical study of Bell Smith Springs Recreation Area in extreme southeastern Illinois is presented. Ecological data are presented for the lowland woods, the slope woods, the bluff tops, and the open fields. An annotated checklist of 700 taxa completes the paper.

Bell Smith Springs Recreation Area is located in northwestern Pope County, Illinois, T 11 S, R 5 E, in extreme southeastern Illinois. The study area is approximately four miles west and slightly north of Eddyville. (See map). The area was named for Bell Smith, the son of the Smith family who settled near the springs (Allen, 1963, p. 325) and who later owned the area. The area is now part of the Shawnee National Forest and maintained by the U. S. Forest Service.

The study area, which comprises 870 acres, is in the Shawnee Hills Section of the Interior Lowland Plateaus Province (Leighton, et al., 1948). The Shawnee Hills are composed of Pounds and Battery Rock sandstone in the Caseyville Formation (Kosanke, et al., 1960). The escarpments of the Caseyville formation mostly face southward; however, the canyon-wall escarpments face in all directions at Bell Smith Springs. The gorge of Bay Creek has been eroded back from the Chester Escarpment. The forces of erosion working on the vertical joints have formed the picturesque features of the area. The joint blocks have fallen into the valley floor from the erosional action of the water. The water erosion has cut a natural bridge which spans 125 feet, 25 feet wide, 30 feet high, and is approximately 8 to 10 feet thick at the apex of the arch.

All of the uplands of southern Illinois are covered by brownish silt known as loess. This material has been deposited by winds from the flood-plains during glacial times. The soil covering the study area is of the Grantsburg-Zanesville soil association. The Grantsburg silt loam is moderately deep, slowly permeable with strong fragipans and covers 20 to 45 per cent of the study area. The Zanesville silt loam is a naturally well-drained acidic soil, low in plant nutrients. It occupies from 25 to 35 per cent of the area. The other soils of the area are shallow to bedrock and are composed of Wellston and Muskingum silt loam (Walter Parks, Jackson County Soil Conservation Service, Personal Communication).

FIGURE 1. — Map of Bell Smith Springs Recreation Area. (Map prepared by K. Weik).

The climate of the Shawnee Hills Section is considered a humid subtropical forest climate (Trewartha, 1943, p. 311). The rainfall distribution appears to be affected by the irregular relief. Warm moist air moving over the flat Coastal Plain Province of the south is forced to rise abruptly as it meets the hills and produces an increase in shower activity (Roberts, et al., 1957, p. 80). Precipitation at New Burnside (near Bell Smith Springs) averages 45.11 inches; an average of 24.62 inches (52 per cent) of rainfall occurs during the growing season, April to September (Page, 1949, p. 358). Even though this rainfall is more than that received in central and northern Illinois, it is less useful for the vegetation because of the tremendous runoff, slowly permeable soils, and the high rainfall-evaporation ratio. The annual temperature at New Burnside averages 57.2° F. with monthly summer norms for June, July, and August of 74.8°F., 78.1° F., and 77.4° respectively (U. S. Department of Commerce, Weather Bureau, Illinois, 1963). The average number of frost-free days for the area totals 187 (Page, 1949).

The study of Bell Smith Springs adds another area in the Shawnee Hills of Illinois to be studied floristically. Previous detailed works have been at Panther's Den (MacMahon, 1960), Giant City State Park (Mohlenbrock, 1954), Devil's Kitchen (Mohlenbrock, et al., 1962), and Ferne Clyffe State Park (Mohlenbrock, et al., 1966). Bell Smith lies further east than any of the other areas previously studied. Although it holds much in common with the other areas, Bell Smith Springs possesses more eastern elements in its flora. Plants such as stonecrop (*Sedum telephioides*), black cohosh (*Cimicifuga rubifolia*), beaked rush (*Rhynchospora capitellata*), rock moss (*Selaginella rupestris*), and others occur at Bell Smith Springs while not apparently at the more westerly stations.

Procedure

Collecting trips, particularly in the early spring, have been made into Bell Smith Springs since 1950. During the summer of 1966, a graduate class in Botany from Southern Illinois University, under the direction of Dr. Robert H. Mohlenbrock, made an intensive study of the area. The study included a search of the entire area for species content and quadratting of the major habitat types to determine vegetation percentages.

Students participating in the study and in the preparation of this manuscript are Donald Abb, Thomas Elias, Paul Fantz, Al Farr, Roy Heidinger, Mike Henshaw, William Hopkins, Gerrit Kloek, Donald Naylor, Roscoe Paugh, Joseph Scott, and Steve Wunderle.

Nomenclature used in this paper generally follows that in Mohlenbrock and Voigt (1959).

The quadratting was done by the random pairs technique developed by Cottam and Curtis (1949). This technique quickly permits the user to record a multitude of data. This method applies the use of a straight line through the study area. At each twenty paces along this straight line, meter quadrats were taken. Within each square-meter area, the total basal area of the herbaceous layer and the per cent composition of each species were determined.

After taking the meter quadrat, the nearest tree with a dbh of more than four inches was identified, measured, and recorded. A second tree, outside an exclusion angle of 180° when facing the first tree, was selected and likewise noted. The distance between the two trees was recorded. A line transect one meter wide between the first and second trees was used to record trees and shrubs with a dbh of one to four inches. With the use of this informa-

tion, the following data were ascertained: the total basal area of each tree and shrub species, the relative basal area (or dominance) of each tree and shrub species, the density of all trees together, the density of each species of tree, and the relative density of each tree and shrub species. Detailed ecological data were obtained for the wooded slopes, the stream banks, the flood-plains, the dry bluffs, and the open fields.

THE WOODED SLOPES

The wooded slopes occupy that area below the bluff top and abandoned fields and the area above the exposed sandstone and bottomland. The soils of the slopes are primarily of two types. The Zanesville silt loam has approximately 20 to 40 inches of loess over bedrock and occurs on slopes of 7 to 18 per cent. The Wellston silt loam has less than 20 inches of loess over solid rock and is found on slopes of 12 to 30 per cent. The available water-supply capacity is low because the air and water move freely through the soil.

The slopes of Bell Smith Springs are of the oak-hickory association, except for an occasional planting of red pine (*Pinus resinosa*). The slopes are of variable composition, but primarily composed of the dominant white oak (*Quercus alba*), black oak (*Quercus velutina*), red oak (*Quercus rubra*), and, to a lesser extent, the shagbark hickory (*Carya ovata*). These species extend to the bluff top and exposed sandstone only to be replaced by the post oak (*Quercus stellata*) and black jack oak (*Quercus marilandica*).

The mid-layer is comprised primarily of white oak, shagbark hickory, sassafras (*Sassafras albidum*), slippery or red elm (*Ulmus rubra*), winged elm (*Ulmas alata*), and an occasional red cedar (*Juniperus virginiana*). However, the dominance of the understory changes with the location of the stations.

The herbaceous layer is variable in composition. The size of plants, variety of species, and amount of accumulated litter determine the frequency of the vegetation. The best represented herb of the slopes was *Panicum boscii*. This panicum had a 52.6 per cent frequency of occurrence in each quadrat and represented 8.2 per cent of the total composition. Other herbs which are conspicuous and generally characteristic of the slopes included poverty oat-grass (*Danthonia spicata*), goldenrods (*Solidago spp.*), Virginia creeper (*Parthenocisus quinquefolia*), pussy-toes (*Antennaria plantaginifolia*), and poison ivy (*Rhus radicans*).

Station one. This area was a north-facing slope which runs east and west. The slope is approximately 400 yards long and 200 yards wide. The soil is rather compact with a moderate covering of leaf litter. The dominant trees are white oak and black oak. The shrub layer consists primarily of shagbark hickory and flowering dogwood (*Cornus florida*) seedlings. The herbaceous layer had an average cover of 3.9 per cent, which consisted chiefly of Virginia creeper, poverty oat-grass, and *Panicum boscii*.

Station two. This area represented an east-facing slope which runs north and south. This station is approximately 300 yards long and 150 yards wide. The soil is rather dry and at the understory vegetation is very sparse with an average cover of 2.8 per cent in the quadrats. The canopy consists primarily of white and black oaks. The understory was chiefly represented by sassafras and shagbark hickory with a small per cent of winged elm, red oak, and black oak. Due to the dry soil, the herbaceous layer was represented by mountain mint (*Cunila origanoides*), *Lespedeza repens*, *Panicum boscii*, and poison ivy.

Station three. This area was a west-facing slope which followed a north-south ridge. The slope was about 800 yards long and varied from 100-200 yards wide. This area had a moderate leaf covering and was dry because of the westerly exposure, but the understory vegetation had a 2.7 per cent average cover in the quadrats. This slope had slightly more ground cover than area two because it received more rainfall during the growing season from the prevailing winds of the southwest. The dominant tree of this slope was white oak, with sugar maple (*Acer saccharum*) the subdominant. The most conspicuous ground cover consisted of seedling white oaks and slippery elms. Herbs which were present included *Panicum boscii*, *P. dichotomum*, Virginia creeper, and cat-brier (*Smilax glauca*).

Station four. Station four had a southern exposure. The area is about 250 yards long and 200 yards in width. This station is rather dry and is primarily composed of planted red pines

(*Pinus resinosa*) near the top of the slope, grasses on the mid-slope, and a small belt of a post oak-black jack oak community on the lower slope. The dominant trees of this station were red pine and post oak; subdominant trees consisted of black jack oak, white oak, and black oak. The shrub layer was represented by red cedar and sassafras. Poverty oat-grass, *Panicum boscii*, and goldenrods were the most prevalent herbaceous plants.

Station five. This station was of south-southeasterly exposure. The area varied from 100-200 yards in length to 200 yards in width. The soil was rather rocky with little available moisture near the surface. Little leaf litter was available to hold much moisture. Consequently, this station had the lowest average cover in the quadrats, 2.1 per cent. White oaks and shagbark hickories were the dominant trees. The shrub layer was comprised chiefly of small shagbark hickories and slippery elms. Examination of the herbaceous layer indicated a predominance of sunflower (*Helianthus decapetalus*), pussy-toes, and *Panicum boscii*.

THE STREAM BEDS AND BANKS

Four streams in the area were investigated. The area between the confluences of Bay Creek and Spring Branch, and Bay Creek and Hunting Branch, however, were much disturbed and have not been included in the ecological analysis of the stream banks. Many vascular plants were found to be common along all the streams studied. Such plants characteristic of this area included panic grasses (*Panicum polyanthes* and *P. microcarpon*), wild hydrangea (*Hydrangea arborescens*), sea oats (*Uniola latifolia*), river birch (*Betula nigra*), smooth alder (*Alnus serrulata*), sensitive plant (*Cassia nictitans*), sycamore (*Platanus occidentalis*), and buttonbush (*Cephalanthus occidentalis*).

Spring Branch was composed of smooth sandstone boulders ranging in diameter from four to twelve inches. The wooded slope generally extended to the stream bank. Very little vegetation was growing in the stream bed because of the rapidly flowing water between and under the rocks. The stream banks were dominated by *Panicum polyanthes* and sea oats. Found in lesser abundance were Christmas fern (*Polystichum acrostichoides*), orange-spotted touch-me-not (*Impatiens biflora*), and pokeweed (*Phytolacca americana*).

Entering each major stream bed was a dry creek which carries water in periods of excessive rainfall. Smooth sandstone boulders to over twelve inches in diameter were also found here. The bank vegetation was similar to Spring Branch except vegetation was found growing among the boulders. Plants common among the boulders were *Panicum polyanthes*, alumroot (*Heuchera parviflora*), cream violet (*Viola striata*), wild bergamot (*Monarda fistulosa*), sensitive plant, and Virginia creeper (*Parthenocisus quinquefolia*). Although the dry stream bed can be found in all the four streams, it is most characteristic of Hunting Branch.

The Bay Creek stream bed and flood-plain were larger than the other streams. Bay Creek from the confluence of Hunting Branch downstream and much of Bay Creek above the confluence of Spring Branch consisted of pools varying in length up to 40 yards. These pools were characterized by banks of soil or rock which rose abruptly two to five feet to the level flood-plain. Neither the pool nor the bank supported much vegetation. The only plants associated directly with these pools were *Carex virescens*, *Carex vulpinoidea*, and water willow (*Dianthera americana*), which grew along the margins of the tail waters.

Large bolders, logs, and debris from high water periods created small islands in between the pools. These areas, although within the stream bank, sometimes rose almost to the level of the flood-plain. The species of trees which dominated these islands were river birch, smooth alder, and sycamore. These seedling trees were growing very densely up to ten feet in height in some areas. In other locations, however, they were scattered and the sycamores grew to heights of 30 feet or more. Commonly associated with these trees were such plants as buttonbush, poison ivy, Virginia creeper, common ragweed (*Ambrosia artemisiifolia*), flowering spurge (*Euphorbia corollata*), pokeweed, Joe-pye-weed (*Eupatorium purpurcum*), common evening primrose (*Oenothera biennis*), and *Panicum polyanthes*.

Occasionally the stream banks were interrupted by abrupt ledges as high as 30 feet or more. These ledges were conspicuously inhabited by wild hydrangea and alumroot.

THE LOWLAND WOODS

The lowland woods are considered to extend from the stream bank to the base of the cliffs or to the beginning of the wooded slopes. In the areas of Hunting Branch and Spring Branch, the lowland woods scarcely existed except at the lower end of the stream near Bay Creek. All areas had the same general profile, a dense canopy of mature trees, little or no understory except seedling trees, a sparse herbaceous layer, and an exposed forest floor.

Station one. (Tables 1 and 2). This station was located along lower Bay Creek and possessed the densest layer with a total basal area of approximately 12 per cent. The dominant herbs of the area were goosegrass (*Galium aparine*), Virginia creeper, cream violet, and butterfly violet (*Viola papilionacea*). Two trees dominated the area, the tulip tree (*Liriodendron tulipifera*) and slippery elm. The shrub layer was well represented by spicebush and sugar maple seedlings.

The other four stations (2-5) were floristically fairly similar. The dominant trees of these stations were the beech and sugar maple, having a frequency of occurrence of 50 to 71 per cent and 14 to 50 per cent, respectively. White oaks and several species of hickories were also common.

Seedling sugar maples dominated the shrub layer in all but lower Bay Creek. Other characteristic vegetation of the shrub layer included young flowering dogwood (*Cornus florida*), beech, and tulip tree seedlings. The herbaceous vegetation had an average cover of 3 to 4 per cent at stations 2-5. Virginia creeper and poison ivy were well represented in each area. Other common herbs included *Desmodium nudiflorum*, bedstraw (*Galium concinnum*), woolly blue violet (*Viola sororia*), cat-brier, wild hydrangea, and *Panicum microcarpon*.

THE DRY BLUFFS

One of the outstanding features of the Shawnee Hills is the dry bluff tops and their canyon wall escarpments. Along these dry bluff tops and rock ledges many habitats and micro-habitats may be found. Bell Smith Springs presents a large array of dry bluff tops and rock ledges.

Many of the cliffs face southward, giving a somewhat more xeric exposure and a better development of nearly bare ledges than would be likely with any other direction of dip except northeastward (Winterringer and Vestal, 1956). The major vegetation is very shrubby due to the sparsity of water and the thinness of the soil. The soil is Muskingum stony silt loam which is a grayish-brown stony silt loam, 0-10″ in depth. It often contains rocks on the surface and mixed through the soil. The substrata are sandstone bedrock, usually more or less broken on the upper portion.

The exposed rock ledges in the area vary from approximately one foot to more than 100 feet in width. In the smaller areas lichens are the predominant members of the flora; however, in the larger areas, small islands of black jack oak and red cedar may be found. Often these small islands of shrubby trees are connected by a thin line of soil and vegetation running along a rock ledge. In many places the height of the bluff is dramatized by the fact that it is too low to overtop the trees rooted in the talus just below the base of the cliff. However, the trees do not reduce the exposure of the ledge to the sun and the wind in most cases

In a number of places large blocks of sandstone have broken away from the main cliff. The blocks often become completely dissociated from the main cliff, often becoming covered with vegetation.

The vegetation on the summit of the sandstone escarpment in the aboriginal zone consists mainly of mats of lichens (*Parmelia spp., Cladonia spp.*) and mosses (*Grimmia spp.*). Such forms occur on exposed ledges and rock slopes. These species help prepare the habitat for other plants which will eventually crowd out their predecessors.

Where thin soil areas occur, such plants as rock moss (*Sedum pulchellum*), pineweed (*Hypericum gentianoides*), flower-of-an-hour (*Talinum parviflorum*), and pencil flower (*Stylosanthes biflora*) are prevalent, as well as the more succulent flowering plants such as cactus (*Opuntia rafinesquii*) and American aloe (*Agave virginica*). The rare small club moss (*Selaginella rupestris*) was found associated with *Cladonia* spp. and the cactus.

As the soil deepens, other plants become conspicuous. Among these are poverty oat-grass, broomsedge (*Andropogon virginicus*), *Crotonopsis elliptica*, stone mint (*Cunila origanoides*), cat-

brier, pussy-toes, and *Panicum* spp. This deep layer usually forms a distinct line of demarcation along the bluff top. This line may be broken by islands of deeper soil especially in shallow crevices and depressions.

Adjacent to the true slopes, the scrubby xerophytic forest abounds. This area consists of such dominant species as red cedar, post oak, black jack oak, and white oak. The trees in this zone are relatively sparse, numbering approximately 100 per acre (Tables 3 and 4).

The shrub layer contains mainly seedlings of the dominant trees as well as sparkleberry (*Vaccinium arboreum*) and hickories (*Carya* spp.).

THE OPEN FIELDS

The open fields are characterized by their lack of fertility and their tendency to erode rapidly. The fields are generally of the Grantsburg silt loam type having 6 to 8 feet over sandstone, shale bedrock, or residium. The surface is of brown silt loam 6-10″ thick. The yellowish-brown silt loam of the subsurface is 4-8″ thick. The yellowish-brown silty clay loam of the subsoil is 12-18″ thick. The silt pan or fragipan is comprised of brown silt loam with gray mottles 12-36″ thick and is very hard when dry. The substrata are brown and yellowish-brown mottled with gray silt loam 0-36″ thick over bedrock residium (Walter Parks, personal communication).

The fields in the Bell Smith Springs area are small and not under cultivation. Many of the abandoned fields have been reforested by the United States Forest Service. The fields are better suited for wildlife food-plots than for cropland or pasture.

Broomsedge is the dominant plant of the fields, with sassafras and dwarf sumac (*Rhus copallina*) the dominant shrubs. Fields that have been reforested have been planted predominantly with white pine. Trees that invade the open fields rarely reach a diameter of more than 4″ due to the dryness of the fields and competition from other species.

The field vegetation in general consists mainly of broomsedge, giving the fields the appearance of a prairie. Other common plants include sassafras, dwarf sumac, cat-brier, partridge pea (*Cassia fasciculata*), and species of *Desmodium*.

Due to a greater disturbance in the roadside fields, there was a marked difference in the vegetation found here in contrast to the open fields. The roadside fields (Table 5) frequently have horsetail milkweed (*Asclepias verticillata*), purple milkweed (*Asclepias purpurascens*), field bindweed (*Convolvulus sepium*), rose gentian (*Sabatia angularis*), and St. John's-wort (*Hypericum perforatum*). Near the edge of the fields, flowering dogwood (*Cornus florida*), redbud (*Cercis canadensis*), and species of oak can be found. Other characteristic plants of this area are dodder (*Cuscuta gronovii*), growing coiled around such plants as the flowering spurge (*Euphorbia corollata*).

The open fields had a vegetation similar to the general field vegetation as described above (Table 5). In addition, there were large quantities of panic grasses (*Panicum* spp.), rough-leaved american feverfew (*Parthenium integrifolium*), common cinquefoil (*Potentilla simplex*), and bush clover (*Lespedeza* spp.). In the more moist areas of the field, the common field rush (*Juncus tenuis*) is often encountered. Near the area where the quadrats were taken, a rare white-flowered rose gentian (*Sabatia angularis f. albiflora*) was found.

Annotated Checklist of Vascular Plants

The total number of taxa of vascular plants thus far recorded from Bell Smith Springs is 700. In similar floristics studies completed in similar topographic regions of the Shawnee Hills, the numbers of vascular plants have been 535 for Panther's Den (Union County), 701 for Ferne Clyffe State Park (Johnson County), and 820 for Giant City State Park (Jackson and Union Counties). While the number for Bell Smith Springs is less than that for Ferne Clyffe and Giant City, the percentage of plants considered rare for southern Illinois is greater at Bell Smith Springs.

Uncommon plants for southern Illinois and found at Bell Smith Springs include *Lycopodium lucidulum*, *L. porophilum*, *Selaginella rupestris*, *Sphenopholis nitida*, *Carex aggregata*, *C. annectens*, *C. careyana*, *C. digitalis*, *C. torta*, *Rhynchospora capitellata*, *R. glomerata*, *Goodyera pubescens*, *Tipularia discolor*, *Talinum parviflorum*, *Cimicifuga rubifolia*, *Dicentra canadensis*, *Sedum telephioides*, *Itea virginica*, *Geum virginianum*, *Desmodium nuttallii*, *Angelica venenosa*, *Heracleum lanatum*, *Triosteum angustifolium*, *Pen-*

stemon dcamii, *Lonicera flavescens*, and *Valeriana pauciflora*.

Twenty-eight taxa are ferns or fern allies while three are gymnosperms (including two introductions). Of the flowering plants, 163 are monocots and 506 are dicots. The largest family is the Compositae with 79 taxa, followed by the Gramineae with 73, the Leguminosae with 48, and the Cyperaceae with 38.

Sixty-six species (about 10%) are considered introduced and naturalized.

Lycopodiaceae

Lycopodium lucidulum Michx. Damp shaded bluff, along Bay Creek. One station found.

Lycopodium porophilum Lloyd & Underw. On shaded sandstone ledge. One station found.

Selaginellaceae

Selaginella apoda (L.) Fern. Wet soil near stream. One colonly found.

Selaginella rupestris (L.) Spring. Exposed sandstone cliff. One colony found.

Isoetaceae

Isoetes butleri Engelm. In moist pockets on sandstone bluff tops; not common, but easily overlooked

Equisetaceae

Equisetum hyemale L. Along Hunting Branch. One colony found.

Ophioglossaceae

Botrychium dissectum Spreng. var. *obliquum* (Muhl.) Clute. Occurs sparingly in both flood-plain woods and slope woods.

Botrychium virginianum (L.) Sw. Lowland and slope woods throughout the area.

Ophioglossum vulgatum L. Flood-plain woods. Found only in one area.

Hymenophyllaceae

Trichomanes boschianum Sturm ex Bosch. Not in study area but found less than a half mile southwest of area beneath overhanging sandstone cliff along Bay Creek.

Polypodiaceae

Adiantum pedatum L. Lowland woods throughout the area.

Asplenium pinnatifidum Nutt. Crevices of dry sandstone walls. Highly variable. A crested specimen was also found.

Asplenium platyneuron (L.) Oaks ex D.C. Eaton. Flood-plain, slope, and upland woods; sparingly found on bluff tops in shade of scrub oaks.

Asplenium rhizophyllum L. On boulders in shaded woods; occasional.

Asplenium trichomanes L. Crevices of moist sandstone cliffs; locally abundant.

Athyrium filix-femina (L.) Bernh. Occasionally in woods near or on sandstone cliffs.

Athyrium pycnocarpon (Spreng.) Tidestrom. Flood-plain woods. One nice colony found.

Athyrium thelypterioides (Michx.) Desv. Flood-plain woods along Bay Creek; junction of lowland woods and slope woods along Spring Branch.

Cheilanthes lanosa (Michx.) DC. Eaton in Torr. Occasional atop dry sandstone bluffs.

Cystopteris fragilis (L.) Bernh. var. *protrusa* Weatherby. Found in nearly all moist, shaded situations.

Dryopteris carthusiana (Fuchs) Villars. On shaded sandstone cliffs; sparingly found.

Dryopteris marginalis (L.) Gray. In shaded woods or on shaded sandstone throughout the area.

Onoclea sensibilis L. Low ground along Bay Creek. One colony found.

Polypodium polypodioides (L.) Watt. On exposed or shaded sandstone; occasional.

Polypodium virginianum L. On sandstone boulders and cliffs, usually in the shade; occasional.

Polystichum acrostichoides (Michx.) Schott. Lowland or slope woods throughout the area.

Thelypteris hexagonoptera (Michx.) Slosson. Lowland woods throughout the area, but not particularly common.

Woodsia obtusa (Spreng.) Torr. Lowland and slope woods or even on exposed sandstone bluff tops; occasional.

Pinaceae

**Pinus resinosa* Ait. Occurs in several forest plantings and showing evidence to re-seed itself.

**Pinus strobus* L. Sparingly planted; there is no evidence that this species is spreading.

Curpressaceae

Juniperus virginiana L. On sandstone bluffs, in slope woods, and along edges of fields; common.

Typhaceae

Typha latifolia L. Around small pond; only one colony found.

Gramineae

Agrostis alba L. Waste ground, roadsides, and fields; common.

Agrostis elliottiana Schult. Dry bluff tops throughout the area, but not common.

Agrostis hyemalis (Walt.) BSP. Common along all streams.

Alopecurus carolinianus Walt. Open field, in moist areas.

Andropogon elliottii Chapm. Along edge of woods; found in three or four locations.

Andropogon virginicus L. Common in fields; also on exposed bluff tops.

Aristida dichotoma Michx. Along edge of woods; only one station recorded.

Aristida oligantha Michx. On exposed sandstone cliffs; not common.

Arundinaria gigantea (Walt.) Chapm. Common in flood-plain along Bay Creek.

Brachyelytrum erectum (Schreb.) Beauv. Near base of slope, in woods, along Spring Branch.

Bromus ciliatus L. In slope woods throughout the area.

**Bromus commutatus* Schrad. Common as a roadside weed.

Bromus purgans L. Occasional in flood-plain woods.

**Bromus racemosus* L. Along a road; one plant found.

**Bromus secalinus* L. In open fields and along roads throughout the area.

**Bromus tectorum* L. In open fields and along roads throughout the area.

Cinna arundinacea L. In flood-plain woods throughout the area.

**Dactylis gromerata* L. Only one station found, that along the edge of a woods on a sandstone bluff.

Danthonia spicata (L.) Beauv. ex Roem. & Schultes. The common grass on the dry, exposed sandstone bluff tops.

Diarrhena americana Beauv. Rich woods, sometimes on shaded boulders.

**Digitaria ischaemum* (Schreb.) Muhl. Along roadside; found occasionally.

**Digitaria sanguinalis* (L.) Scop. Found in most weedy situations.

**Echinochloa pungens* (Poir.) Rydb. Occasionally found in moist weedy areas.

**Eleusine indica* (L.) Gaertn. In a grassy field.

Elymus canadensis L. Edge of woods; roadsides; edge of fields.

Elymus villosus Muhl. ex. Willd. Edge of woods; roadsides.

Elymus virginicus L. Lowland woods; roadsides; open fields; common.

**Eragrostis cilianensis* (All.) Link ex Lutati. In an open field.

Eragrostis frankii C.A. Mey. in Steud. Edge of woods on sandstone bluff.

Eragrostis pectinacea (Michx.) Nees. Found in most weedy areas.

Eragrostis spectabilis (Pursh) Steud. On sandstone bluffs; roadsides.

**Festuca elatior* L. Along a road; only found once.

Festuca obtusa Bieler. Low woods along Spring Branch; also in slope woods.

Festuca octoflora Walt. var. *tenella* (Willd.) Fern. Common on exposed bluff tops.

Glyceria striata (Lam.) Hitchc. Along streams and in low woods.

**Hordeum jubatum* L. Waste areas.

Hordeum pusillum Nutt. Open fields and along roads; common.

Hystrix patula Moench. Lowland and slope woods; occasional.

Leersia virginica Willd. Woods along Bay Creek.

Melica mutica Walt. Edge of woods on sandstone bluff.

Melica nitens (Scribn.) Nutt. ex Piper. Exposed sandstone bluff top.

Muhlenbergia sobolifera (Muhl.) Trin. Slope woods; one of the more common woodland grasses.

Panicum anceps Michx. Woods at edge of sandstone bluff; growing in a moist depression.

Panicum barbulatum Mich. Woods at edge of sandstone bluff.

Panicum boscii Poir. in Lam. Flood-plain woods; along all streams.

Panicum clandestinum L. Along Bay Creek in several places.

Panicum depauperatum Muhl. Edge of woods of sandstone bluff.

Panicum dichotomiflorum Michx. Along all streams.

Panicum dichotomum L. Slope woods; woods on sandstone bluffs.

Panicum huachucae Ashe. Common in fields; occasional along the edge of woods.

Panicum latifolium L. Slope woods; occasional.

Panicum linearifolium Scribn. ex Britt. & Brown. Woods on sandstone bluff top.

Panicum microcarpon Muhl. ex Ell. Flood-plain woods; common.

Panicum polyanthes Schult. Along most of the streams.
Panicum praecocius Hitchc. & Chase. Dry ground on sandstone bluff top.
Panicum sphaerocarpon Ell. Woods of sandstone bluff.
Panicum tennesseense Ashe. Along Bay Creek; not common.
Panicum sphaerocarpon Ell. Woods of sandstone bluff tops; common.
Paspalum laeve Michx. Along roads; occasional.
**Phleum pratense* L. Fields and along roads; common.
**Poa annua* L. Moist waste ground; occasional.
Poa chapmaniana Scribn. Dry areas in fields; not common.
**Poa compressa* L. Waste ground and occasionally in woods.
**Poa pratensis* L. Common in nearly all habitat types.
Poa sylvestris A. Gray. Lowland woods; not common.
**Setaria faberi* Herrm. Roadsides and fields; common.
**Setaria lutescens* (Weigel) F.T. Hubb. Roadsides and fields; common.
**Setaria viridis* (L.) Beauv. Fields; common.
**Sorghum halepense* (L.) Pers. Roadsides; occasional.
Sphenopholis nitida (Bieler) Scribn. Woods on sandstone bluff tops.
Sphenopholis obtusata (Michx.) Scribn. Woods along roads.
Triodia flava (L.) Smyth. Fields and roadsides; common.
Uniola latifolia Michx. Lowland and slope woods; common.

Cyperaceae

Bulbostylis capillaris (L.) C.B. Clarke in Hook. f. Moist depressions on dry sandstone bluffs.
Carex aggregata Mack. Dry woods; only one colony found. This species is known in Illinois also from Adams and Madison Counties.
Carex albursina Sheldon. Lowland woods; occasional. This species has the widest leaves of any species of *Carex* in Illinois.
.Carex annectens Bickn. Moist area along a road; only one plant found. This Pope County collection marks only the fifth county in Illinois for this species.
Carex artitecta Mack. Mostly dry woods; common. One of the earliest species of *Carex* to bloom.
Carex blanda Dewey. Moist or dry woods; common.
Carex bushii Mack. Dry woods; fields; common.
Carex careyana Torr. ex Dewey. Deep lowland woods; not common.
Carex cephalophora Muhl. ex Willd. Along roads; in fields; common.
Carex convoluta Mack. One colony found half-way up a wooded slope beneath pignut hickory and white oak.
.Carex digitalis Willd. Lowland woods beneath beech and sugar maple; rare. Only a station in Hardin County is known besides this one.
Carex frankii Kunth. One station along Bay Creek.
Carex glaucodea Tuckerm. ex Olney. Dry woodlands, frequently on sandstone bluffs; occasional.
Carex granularis Muhl. ex Willd. Wooded slopes; occasional.
Carex grisea Wahl. Woods near streams; occasional.
Carex hirsutella Mack. Dry woods, especially on sandstone bluffs; common.
Carex jamesii Schw. Woods on sandstone bluffs.
Carex lupulina Muhl. ex Willd. Low area along a road; only one colony found.
Carex lurida Wahl. Low area along a road, growing with *C. lupulina.*
Carex muhlenbergii Schk. Dry woodlands; occasional.
Carex retroflexa Muhl. ex Willd. Dry woods; occasional.
Carex rosea Schk. ex Willd. Lowland woods; occasional.
Carex squarrosa L. In stream bed of Spring Branch.
Carex torta Boot ex Carey in Gray. In rapid-flowing waters of Bay Creek.
Carex tribuloides Wahl. Moist ditch along a road; only a single colony found.
Carex umbellata Schk. in Willd. On open sandstone boulders; not common. This is one of the earliest flowering species of *Carex* in Illinois.
Carex virescens Muhl. ex Willd. Lowland woods near Bay Creek; not common.
Carex vulpinoidea Michx. Along all the streams; common.
Cyperus aristatus Rottb. Moist depressions on sandstone bluff tops.
Cyperus esculentus L. Waste areas; along streams; common.
Cyperus ovularis (Michx.) Torr. Bluff tops; open fields; roadsides; common.

Cyperus strigosus L. Fields; along streams; common.
Eleocharis acicularis (L.) Roem. & Schultes. In moist depressions on sandstone bluff top.
Eleocharis obtusa (Willd.) Schultes. Along most of the streams.
Rhynchospora capitellata (Michx.) Vahl. Along Bay Creek; rare.
Rhynchospora glomerata (L.) Vahl. Along sandy creek; rare.
Scirpus atrovirens Willd. In moist, open areas; common.
Scirpus lineatus Michx. Wet areas along roads; occasional.

Araceae

Arisaema dracontium (L.) Schott. Lowland woods; occasional.
Arisaema triphyllum (L.) Schott in Schott & Endl. Lowland woods; common.

Commelinaceae

Commelina communis L. Along Bay Creek; occasional.
**Commelina diffusa* Burm. f. Along several of the streams; occasional.
Tradescantia ohiensis Raf. Along a road; only one colony found.
Tradescantia subaspera Ker-Gawler. Lowland or slope woods; occasional.
Tradescantia virginiana L. Open woods; fields; common.

Juncaceae

Juncus acuminatus Michx. Along Bay Creek; occasional.
Juncus biflorus Ell. Eroded gulley in field.
Juncus dudleyi Wieg. In an open field; found only once.
Juncus interior Wieg. On sandstone bluff; rare.
Juncus marginatus Rostk. Along a creek; rare.
Juncus tenuis Willd. Along streams; on sandstone bluffs; in open fields; common.
Luzula echinata (Small) Hermann. Dry woods, associated with sandstone outcroppings; occasional.
Luzula multiflora (Retz.) Lejeune. Woods on sandstone bluffs; occasional.

Liliaceae

Allium canadense L. Waste ground; along roads; on sandstone bluff tops.
**Allium vineale* L. Waste ground; common.
**Asparagus officinalis* L. Along roads; occasional.
Erythronium albidum Nutt. Moist shaded woods; occasional.
Erythronium americana Ker. Moist shaded sandstone ledges.
Nothoscordum bivalve (L.) Britt. in Britt. & Brown. Fields; on dry sandstone bluff tops.
Polygonatum biflorum (Walt.) Ell. Mossy slope near creek; on sandstone bluff tops; occasional.
Polygonatum commutatum (J. H. Schultes) Dietr. ex Otto & Dietr. Woods; common.
Smilacina racemosa (L.) Desf. Woods; common.
Smilax bona-nox L. In woods and climbing on trees on sandstone bluff tops; occasional.
Smilax glauca Walt. Woods; often on bluff tops; common.
Smilax hispida Muhl. ex Torr. Woods; common.
Smilax lasioneuron Hook. Lowland and slope woods; occasional.
Smilax pulverulenta Michx. Lowland woods; occasional.
Smilax rotundifolia L. Woods, particularly along Spring Branch; occasional.
Trillium gleasonii Fern. Deep, shaded woods; not common.
Trillium recurvatum Beck. Woods; common.
Uvularia grandiflora Sm. Deep, shaded woods; occasional.
Uvularia sessilifolia L. Moist woods; rare.

Dioscoreaceae

Dioscorea quaternata [Walt.] J.F. Gmel. Along most streams; lowland woods; common.
Dioscorea villosa L. Slope woods; occasional.

Amaryllidaceae

Agave virginica L. On exposed sandstone bluff tops; occasional.
Hypoxis hirsuta (L.) Coville. Slope woods; bluff top woods; occasional.

Iridaceae

Sisyrinchium albidum Raf. Open areas near streams; occasional.
Sisyrinchium angustifolium Mill. Fields; bluff tops; occasional.

Orchidaceae

Aplectrum hyemale (Muhl.) Torr. Lowland woods; occasional.
Corallorhiza odontorhiza (Willd.) Nutt. Lowland woods; rare.
Corallorhiza wisteriana Conrad. Lowland and slope woods; rare.

Goodyera pubescens (Willd.) R.Br. ex Ait. Lowland woods; rare.
Habenaria peramoena Gray. Along Bay Creek; only one plant found.
Liparis lilifolia (L.) Rich. ex Lindl. Colony under *Juniperus virginiana* near edge of woods on sandstone bluff.
Orchis spectabilis L. Lowland woods; rare.
Spiranthes cernua (L.) Rich. Edge of field; one colony found.
Spiranthes gracilis (Bigel.) Beck. Mossy wooded slopes; rare.
Spiranthes grayi Ames. In thin soil on sandstone bluff tops; occasional.
Tipularia discolor (Pursh) Nutt. Moist, shaded woods; rare.

Salicaceae

**Populus alba* L. One specimen persistent from cultivation was found.
Populus deltoides Marsh. Along all streams; common.
Salix amygdaloides Anders. Along Hunting Branch; rare.
Salix interior Rowlee. Along a few of the streams; occasional.
Salix nigra Marsh. Along all streams; common.
Salix sericea Marsh. Along small tributary to Bay Creek; rare.

Juglandaceae

Carya cordiformis (Wang.) K. Koch. Primarily in slope woods; occasional.
Carya glabra (Mill.) Sweet. In slope and bluff top woods; common.
Carya ovalis (Wang.) Sarg. Slope woods; occasional.
Carya ovata (Mill.) K. Koch. Lowland and slope woods; common.
Carya texana Buckl. On sandstone bluff tops; rare.
Carya tomentosa (Poir.) Nutt. Lowland and slope woods; rare.
Juglans cinerea L. Rich, mesic woods; rare.
Juglans nigra L. Lowland and slope woods; occasional.

Betulaceae

Alnus serrulata (Ait.) Willd. Along most of the streams; common.
Betula nigra L. Along most of the streams; occasional.
Carpinus caroliniana Walt. Lowland and slope woods; common.
Corylus americana Walt. Lowland and slope woods, particularly near streams; common.
Ostrya virginiana (Mill.) K. Koch. Lowland and slope woods; common.

Fagaceae

Fagus grandifolia Ehrh. Low, mesic woods; common.
Quercus alba L. Most woodland types; common.
Quercus bicolor Willd. ex Muhl. Along a stream; only one tree found.
Quercus coccinea Muench. Edge of woods at upper parking lot; only one tree found.
Quercus falcata Michx. Slope woods; rare.
Quercus imbricaria Michx. Slope and bluff top woods; occasional.
Quercus marilandica Muench. Bluff top woods; common.
Quercus michauxii Nutt. Along Bay Creek; rare.
Quercus muhlenbergii Engelm. Slope woods; occasional.
Quercus palustris Muench. Along a few of the streams; occasional.
Quercus rubra L. Most woodland types; common.
Quercus stellata Wang. Bluff top woods; common.
Quercus velutina Lam. Most woodland types; common.

Ulmaceae

Celtis occidentalis L. Lowland woods; occasional.
Celtis pumila Pursh. Woods on sandstone bluffs; common.
Ulmus alata Michx. Woods on sandstone bluffs; common.
Ulmus americana L. Most woodland types; common.
Ulmus rubra Muhl. Primarily in flood-plain woods; common.

Moraceae

Morus rubra L. Lowland woods; occasional.

Urticaceae

Boehmeria cylindrica (L.) Sw. Flood-plain woods; common.
Laportea canadensis (L.) Gaudich. Flood-plain woods; occasional.
Parietaria pennsylvanica Muhl. ex Willd. In dry, powdery soil beneath sandstone cliffs; occasional.
Pilea pumila (L.) Gray. Flood-plain woods; along streams; common.

Aristolochiaceae

Aristolochia serpentaria L. Deep, moist woods; occasional, but often overlooked.
Asarum reflexum Bickn. Deep, moist woods; common.

Polygonaceae

Polygonum aviculare L. Edge of fields; occasional.
Polygonum convolvulus L. In fields; occasional.
Polygonum hydropiperoides Michx. Along streams; common.
Polygonum lapathifolium L. Along streams; common.
Polygonum pennsylvanicum L. Along streams; in fields; common.
Polygonum persicaria L. Along roads; edges of fields; common.
Polygonum punctatum Ell. Along streams; along roads in moist areas; common.
Polygonum scandens L. In woods on slopes and bluff tops; occasional.
Polygonum tenue Michx. Dry, exposed sandstone bluff top; occasional.
Polygonum virginianum L. Lowland woods; common.
**Rumex acetosella* L. In fields; along roads; common.
**Rumex crispus* L. Along roads; occasional.

Chenopodiaceae

Chenopodium album L. Field; along roads; common.
Chenopodium standleyanum Aellen. Slope woods; occasional.
Chenopodium hybridum L. Lowland woods; occasional.

Phytolaccaceae

Phytolacca americana L. Edge of woods; common.

Illecebraceae

Paronychia canadensis (L.) Wood. In shade on sandstone bluff tops; rare.
Paronychia fastigiata (Raf.) Fern. In sandy soil at base of cliffs; on sandstone bluffs; occasional.

Aizoaceae

**Mollugo verticillata* L. Moist waste areas; occasional.

Portulacaceae

Claytonia virginica L. Lowland and slope woods; common.
Talinum parviflorum Nutt. ex Torr. & Gray. Exposed sandstone bluff top; rare.

Caryophyllaceae

Cerastium nutans Raf. Along Bay Creek; occasional.
**Cerastium viscosum* L. Found in two areas along streams.
**Cerastium vulgatum* L. Along roads; edges of fields; occasional.
**Dianthus armeria* L. In stream bed of Bay Creek.
Silene antirrhina L. Along a road; rare.
Silene stellata (L.) Ait f. Slope woods; occasional.
**Stellaria media* (L.) Vill. In waste ground; occasional.

Magnoliaceae

Liriodendron tulipifera L. Rich, moist woods; common.

Annonaceae

Asimina triloba (L.) Dunal. Flood-plain and slope woods; common.

Ranunculaceae

Actaea alba (L.) Mill. Lowland woods; common.
Anemone virginiana L. Lowland and slope woods; occasional.
Anemonella thalictroides (L.) Spach. Flood-plain woods; common.
.*Aquilegia canadensis* L. On shaded sandstone; common.
Cimicifuga rubifolia Kearney. Deep, moist woodland; rare.
Delphinium tricorne Michx. Flood-plain and slope woods; occasional.
Hepatica acutiloba DC. Shaded, rocky woods; occasional; one of the first species to flower.
Hydrastis canadensis L. Lowland woods; occasional.
Isopyrum biternatum (Raf.) Torr. & Gray. Lowland and slope woods; common.
Myosurus minimus L. Along streams; occasional.
Ranunculus abortivus L. Along Bay Creek; occasional.
Ranunculus fascicularis Muhl. Slope and bluff top woods; occasional.
Ranunculus hispidus Michx. Slope and bluff top woods; occasional.
Ranunculus micranthus Nutt. ex Torr. & Gray. Slope woods; occasional.
Ranunculus recurvatus Poir. in Lam. Flood-plain woods; occasional.
Ranunculus septentrionalis Poir. in Lam. Flood-plain along Bay Creek; occasional.
Thalictrum dioicum L. Lowland woods; rare.

Berberidaceae

Caulophyllum thalictroides (L.) Michx. Lowland and slope woods; occasional.
Podophyllum peltatum L. In all woodland types; common.

Menispermaceae

Menispermum canadense L. Wooded slopes; occasional.

Papaveraceae

Sanguinaria canadensis L. Lowland woods; common.

Stylophorum diphyllum (Michx.) Nutt. Rich woodlands; occasional.

Fumariaceae

Corydalis flavula (Raf.) DC. Moist, shaded woods; common.

Dicentra canadensis (Goldie) Walp. One colony found on a wooded slope.

Dicentra cucullaria (L.) Bernh. Lowland and slope woods; occasional.

Cruciferae

**Arabidopsis thaliana* (L.) Heynh. Edge of roads; occasional.

Arabis canadensis L. Slope woods; occasional.

Arabis laevigata (Muhl.) Poir. in Lam. All woodland types; common.

Arabis virginica (L.) Poir. in Lam. Along edge of fields; occasional.

**Barbarea vulgaris* R.Br. in Ait. Waste areas; common.

**Capsella bursa-pastoris* (L.) Medic. Along roads; in fields; common.

Cardamine parviflora L. var. *arcnicola* (Britt.) O.E. Schulz. Along streams; occasional.

Cardamine pennsylvanica Muhl. ex Willd. Flood-plain woods; occasional.

Dentaria laciniata Muhl. Lowland and slope woods; common.

Draba brachycarpa Nutt. ex Torr. & Gray. In fields; common.

Lepidium virginicum L. All waste areas; in fields; common.

Rorippa islandica (Oeder) Borbas. Along Hunting Branch; several plants found.

Rorippa sessiliflora (Nutt.) Hitchc. Along most streams; occasional.

Crassulaceae

Penthorum sedoides L. Along streams; occasional.

Sedum pulchellum Michx. On sandstone cliffs and boulders; occasional.

Sedum telephioides Michx. On sand stone cliffs and boulders, usually in the shade; occasional.

Sedum ternatum Michx. Very common in a flood-plain slippery elm woods, otherwise rare.

Saxifragaceae

Heuchera americana L. var. *hirsuticaulis* (Wheelock) Rosend., Butters, & Lak. Along Bay Creek near bases of oak-hickory slopes; occasional.

Heuchera parviflora Bartl. var. *rugelii* (Shuttlw.) Rosend., Butters, & Lak. Shaded sandstone cliffs; common. The flower period extends later into autumn than that of any other species.

Escalloniceae

Itea virginica L. Along Bay Creek; rare.

Hydrangeaceae

Hydrangea arborescens L. Woods and on shaded sandstone; common. This species is extremely variable in leaf pubescence and degree of sterile flowers present.

Hamamelidaceae

Liquidambar styraciflua L. Flood-plain woods; occasional.

Platanaceae

Platanus occidentalis L. Flood-plain woods and along streams; common.

Rosaceae

Agrimonia parviflora Ait. Along several streams; occasional.

Agrimonia pubescens Wallr. Lowland and slope woods; occasional.

Agrimonia rostellata Wallr. Lowland and slope woods; occasional.

Amelanchier arborea (Michx. f.) Fern. On sandstone cliffs; occasional. This species flowers as early as mid-March.

Aruncus dioicus (Walt.) Fern. Rocky, shaded woods; occasional.

Crataegus crus-galli L. Open woods; occasional.

Fragaria virginiana Duch. Along a road; occasional.

Geum canadense Jacq. Lowland and slope woods; along a road; common.

Geum vernum (Raf.) Torr. & Gray. Lowland and slope woods; occasional.

Geum virginianum L. Open woods near upper slope; only one plant found.

Gillenia stipulata (Muhl.) Trel. ex Branner & Coville. Wooded slopes and bluff tops; occasional.

Potentilla simplex Michx. Woods; fields; along streams; common.

Prunus americana Marsh. Open woods; occasional.

Prunus hortulana Bailey. Along roads; occasional.

Prunus serotina Ehrh. Woods; common.

Rosa carolina L. Along roads; wooded slopes; occasional.

Rosa setigera Michx. Wooded slopes; occasional.
Rubus allegheniensis Porter. Wooded slopes; fields; common.
Rubus flagellaris Willd. Wooded slopes; bluff tops; occasional.
Rubus occidentalis L. Fields; occasional.
Rubus ostryifolius Rydb. in Britt. Fields; common.
Rubus pennsylvanicus Poir. Fields; occasional.

Leguminosae

Amphicarpa bracteata (L.) Fern. Wooded slopes; occasional.
Apios americana Medic. Wooded slopes; occasional.
Baptisia leucantha Torr. & Gray. Floodplain along Bay Creek; rare.
Cassia fasciculata Michx. Woods; fields; along streams; roadsides; common.
Cassia marilandica L. Along a road; rare.
Cassia nictitans L. Fields; along streams; occasional.
Cercis canadensis L. Lowland and slope woods; common.
Clitoria mariana L. Woods on sandstone bluff top; occasional.
Crotalaria sagittalis L. Fields; occasional.
Desmodium canescens (L.) DC. Wooded slopes; occasional.
Desmodium ciliare (Muhl.) DC. Wooded slopes; rare.
Desmodium cuspidatum (Muhl.) Loud. Wooded slopes; occasional.
Desmodium glutinosum (Muhl.) Wood. Lowlands woods; occasional.
Desmodium illinoense Gray. Field; rare.
Desmodium marilandicum (L.) DC. Edge of woods near Natural Bridge.
Desmodium nudiflorum (L.) DC. Lowland and slope woods; common.
Desmodium nuttallii (Schindl.) Schubert. Bluff top woods; rare.
Desmodium paniculatum (L.) DC. Wooded slopes; common.
Desmodium pauciflorum (Nutt.) DC. Wooded slopes; occasional.
Desmodium rotundifolium DC. Slope and bluff top woods; occasional.
Desmodium sessilifolium (Torr.) Torr. & Gray. Wooded slopes; occasional.
Galactia volubilis (L.) Britton. Slope and bluff top woods; occasional.
Gleditsia triacanthos L. Along streams; occasional.
Lespedeza capitata Michx. Bluff top woods; occasional.
**Lespedeza cuneata* (Dom.-Cours.) G. Don. Along roads; common.
Lespedeza hirta (L.) Hornem. Bluff top woods; occasional.
Lespedeza intermedia S. Wats. in Britt. Wooded slopes; occasional.
Lespedeza procumbens Michx. Slope and bluff top woods; common.
Lespedeza repens (L.) Bart. Wooded slopes; occasional.
**Lespedeza stipulacea* Maxim. Planted and escaped along roads.
**Lespedeza striata* (Thunb.) Hook. & Arnott. Along roads; fields; common.
Lespedeza stuvei Nutt. Wooded slopes; occasional.
Lespedeza violacea (L.) Pers. Wooded slopes; occasional.
Lespedeza virginica (L.) Britt. Bluff top woods; occasional.
**Medicago sativa* L. Along roads; in fields; occasional.
**Melilotus alba* Desr. Fields; along roads; common.
**Melilotus officinalis* (L.) Lam. Fields; roadsides; common.
Psoralea psoralioides (Walt). Cory. Slope and bluff top woods; occasional.
Strophostyles helvola (L.) Britt. in Britt. & Brown. Along roads; occasional.
Strophostyles leiosperma (Torr. & Gray) Piper. Along Spring Branch; rare.
Strophostyles umbellata (Muhl.) Britt. in Britt. & Brown. Along roads; fields; occasional.
Stylosanthes biflora (L.) BSP. Slope and bluff top woods; occasional.
Tephrosia virginiana (L.) Pers. var. *holosericea* (Nutt.) Torr. & Gray. Slope and bluff top woods; occasional.
**Trifolium hybridum* L. Roadsides; fields; occasional.
**Trifolium pratense* L. Roadsides; fields; common.
**Trifolium procumbens* L. Roadsides; occasional.
**Trifolium repens* L. Fields; roadsides; common.
**Vicia villosa* Roth. Roadsides; occasional.

Geraniaceae

Geranium carolinianum L. Roadsides; fields; common.
Geranium maculatum L. Lowland and slope woods; common.

Oxalidaceae

Oxalis cymosa Small. Roadsides; slope

and bluff top woods; fields; common. Much variation exists in this species.
Oxalis stricta L. Fields; roadsides; wooded slopes; common.
Oxalis violacea L. Slope and bluff top woods; common.

Linaceae

Linum medium (Planch.) Britt. in Britt. & Brown. Exposed sandstone bluff top; occasional.
Linum striatum Walt. Rock barrens along Hill Branch, October 4, 1952, *H.E. Ahles 6931.*
Linum virginianum L. Wooded slopes; occasional.

Balsaminaceae

Impatiens biflora Walt. Flood-plain woods; along streams; common.
Impatiens pallida Nutt. Flood-plain woods; occasional.

Polygalaceae

Polygala ambigua Nutt. Woods on sandstone bluff top; rare.
Polygala sanguinea L. Fields; occasional.

Euphorbiaceae

Acalypha gracilens Gray. Bluff top woods; rare.
Acalypha rhomboidea Raf. Roadsides; fields; occasional.
Acalypha virginica L. Roadsides; fields; open woods; occasional.
Chamaesyce maculata (L.) Small. Roadsides; fields; common.
Chamaesyce supina (Raf.) Moldenke. Fields; roadsides; occasional.
Croton monanthogynus Michx. Exposed sandstone bluff tops; rare.
Crotonopsis elliptica Willd. Exposed sandstone bluff tops; occasional.
Euphorbia corollata L. Slope and bluff top woods; fields; roadsides; common.
Euphorbia obtusata Pursh. Lowland woods; rare.

Celastraceae

Celastrus scandens L. Woods; occasional.
Euonymus atropurpureus Jacq. Lowland woods; occasional.
Euonymus obovatus Nutt. Rich woods; occasional.

Aquifoliaceae

Ilex decidua Walt. Flood-plain woods; occasional.

Anacardiaceae

Rhus aromatica Ait. Slope and bluff top woods; occasional. One of the first shrubs to flower.
Rhus copallina L. Flood-plain woods; roadsides; common.
Rhus glabra L. Wooded slopes; common.
Rhus radicans L. In all habitats; common.

Staphyleaceae

Staphylea trifolia L. Along several streams; common.

Aceraceae

Acer negundo L. Flood-plain woods; occasional.
Acer rubrum L. Flood-plain woods and wooded bluff tops; common.
Acer saccharinum L. Flood-plain woods; common.
Acer saccharum Marsh. Lowland and slope woods; common.

Hippocastanaceae

Aesculus glabra Willd. Woods; occasional.

Rhamnaceae

Ceanothus americanus L. Slope and bluff top woods; common.
Rhamnus caroliniana Walt. Lowland woods; occasional.

Vitaceae

Parthenocissus quinquefolia (L.) Planch. In all habitats; common.
Vitis aestivalis Michx. Flood-plain woods; common.
Vitis cinerea Engelm. ex Millardet. Wooded slopes; old fields; wooded bluff tops; common.
Vitis vulpina L. Along Spring Branch.

Tiliaceae

Tilia americana L. Flood-plain woods along Bay Creek; occasional.

Malvaceae

Sida spinosa L. Fields; occasional.

Hypericaceae

Ascyrum multicaule Michx. Wooded slopes and bluff tops; occasional.
Hypericum drummondii (Grev. & Hook.) Torr. & Gray. Exposed bluff tops; occasional.
Hypericum gentianoides (L.) BSP. Exposed bluff tops; occasional.
Hypericum mutilum L. Along Bay Creek and Spring Branch; occasional.
Hypericum perforatum L. Field; rare.
Hypericum prolificum L. Wooded bluffs; occasional.

Hypericum punctatum Lam. Roadsides; occasional.
Hypericum sphaerocarpon Michx. Roadsides; wooded slopes; occasional.

Cistaceae

Lechea tenuifolia Michx. Bluff top woods; occasional.

Violaceae

Hybanthus concolor (Forst.) Spreng. Lowland woods; common.
Viola eriocarpa Schw. Lowland woods; common.
Viola falcata Greene. Wooded slopes; occasional.
Viola missouriensis Greene. Flood-plain woods; occasional.
Viola papilionacea Pursh. Woods; common.
Viola rafinesquii Greene. Woods; fields; roadsides; common.
Viola sagittata Ait. Wooded slopes; rare.
Viola sororia Willd. Woods; common.
Viola striata Ait. Flood-plain woods; occasional.

Lauraceae

Lindera benzoin (L.) Blume. Lowland and slope woods; common.
Sassafras albidum (Nutt.) Nees. All woodland types; roadsides; edges of fields; common.

Lythraceae

Cuphea petiolata (L.) Koehne. Along a road; rare.
Rotala ramosior (L.) Koehne. Along Hunting Branch; rare.

Passifloraceae

Passiflora lutea L. Lowland and slope woods; occasional.

Cactaceae

Opuntia rafinesquii Engelm. Exposed sandstone bluff tops; occasional.

Cucurbitaceae

Melothria pendula L. Along Bay Creek; rare.

Onagraceae

Circaea latifolia Hill. Flood-plain woods; common.
Ludwigia alternifolia L. Moist woods; occasional.
Ludwigia polycarpa Short & Peter. Roadside; rare.
Oenothera biennis L. Along roads; common.
Oenothera linifolia Nutt. Exposed sandstone bluff tops; rare.
Oenthera pilosella Raf. Along a road; rare.
Oenothera tetragona Roth. Wooded slopes; rare.

Callitrichaceae

Callitriche terrestris Raf. Moist, shaded soil; occasional.

Cornaceae

Cornus alternifolia L. f. Lowland woods; rare.
Cornus drummondii C.A. Mey. Along several streams; common.
Cornus florida L. Woods; common.
Nyssa sylvatica Marsh. Lowland woods; occasional.

Araliaceae

Aralia racemosa L. Shaded sandstone boulders; rare.
Panax quinquefolius L. Lowland woods; occasional.

Umbelliferae

Angelica venenosa (Greenway) Fern. In dried-up stream bed; rare.
Chaerophyllum procumbens (L.) Crantz. Fields; roadsides; common.
Chaerophyllum tainturieri Hook. Fields; roadsides; common.
Cicuta maculata L. Along Bay Creek; rare.
Cryptotaenia canadensis (L.) DC. Flood-plain woods; common.
**Daucus carota* L. Waste ground; common.
Erigenia bulbosa (Michx.) Nutt. Lowland woods; occasional.
Eryngium yuccifolium Michx. Slope and bluff top woods; occasional.
Heracleum lanatum Michx. Flood-plain woods; rare.
Osmorhiza claytonii (Michx.) Clarke in Hook. f. Lowland woods; occasional.
Osmorhiza longistylis (Torr.) DC. Lowland and slope woods; occasional.
Sanicula canadensis L. All woodland types; occasional.
Sanicula gregaria Bickn. Flood-plain and slope woods; occasional
Thaspium trifoliatum (L.) Gray. All woodland types; common. Both the purple- and yellow-flowered forms have been found.

Ericaceae

Monotropa uniflora L. Lowland woods; rare.
Vaccinium arboreum Marsh. Bluff top woods; common.

Vaccinium vacillans Torr. Bluff top woods; occasional.

Ebenaceae

Diospyros virginiana L. Wooded slopes; edge of fields; common.

Primulaceae

Dodecatheon frenchii (Vasey) Rydb. Beneath overhanging sandstone cliffs; locally abundant.

Dodecatheon meadia L. Wooded slopes; common.

Lysimachia ciliata L. Flood-plain woods; common.

Lysimachia lanceolata Walt. Flood-plain woods; common.

Samolus parviflorus Raf. Moist, shaded soil; occasional.

Oleaceae

Forestiera acuminata (Michx.) Poir. in Lam. Along Bay Creek; rare.

Fraxinus americana L. All woodland types; common.

Fraxinus lanceolata Borkh. Lowland woods; occasional.

Fraxinus pennsylvanica Marsh. Lowland woods; rare.

Loganiaceae

Spigelia marilandica L. Bluff top woods; rare.

Gentianaceae

Frasera caroliniensis Walt. Wooded slopes; occasional.

Gentiana saponaria L. Lowland woods; rare.

Obolaria virginica L. Rich lowland woods; rare.

Sabatia angularis (L.) Pursh. Wooded slopes; fields; occasional. The white-flowered form was found mixed with typical specimens.

Apocynaceae

Amsonia tabernaemontana Walt. Along Bay Creek; occasional.

Apocynum cannabinum L. Waste ground; common.

Apocynum sibiricum Jacq. Exposed sandstone bluff; fields; roadsides; common.

Asclepiadaceae

Ampelamus albidus (Nutt.) Britt. Along several streams; common.

Asclepias exaltata L. Lowland woods; rare.

Asclepias incarnata L. Along some streams; occasional.

Asclepias purpurascens L. Fields; occasional.

Asclepias syriaca L. Roadsides; occasional.

Asclepias tuberosa L. Wooded slopes; fields; roadsides; common.

Asclepias variegata L. Wooded slopes; rare.

Asclepias verticillata L. Bluff top woods; field; occasional.

Convolvulaceae

Convolvulus americanus (Sims) Greene. Fields; roadsides; common.

Convolvulus arvensis L. Roadsides; occasional.

Cuscuta cuspidata Engelm. Roadside near field; one station found.

Cuscuta gronovii Willd. ex Roem. & Schultes. Roadside; growing on *Euphorbia corollata.*

**Ipomoea hederacea* Jacq. Slope woods; fields; common.

Ipomoea lacunosa L. Along Bay Creek; occasional.

Ipomoea pandurata (L.) Meyer. Fields, roadsides; occasional.

Polemoniaceae

Phlox bifida Beck. Bluff top woods; occasional.

Phlox divaricata L. Lowland and slope woods; common.

Phlox glaberrima L. Lowland woods at base of slope; rare.

Phlox paniculata L. Flood-plain woods; along streams; occasional.

Pholox pilosa L. Wooded slopes; occasional.

Polemonium reptans L. Lowland and slope woods; common.

Hydrophyllaceae

Hydrophyllum appendiculatum Michx. Lowland woods; occasional.

Hydrophyllum canadense L. Lowland woods; rare.

Phacelia bipinnatifida Michx. Lowland woods; common.

Phacelia purshii Buckley. Lowland woods; occasional.

Boraginaceae

Cynoglossum virginianum L. Wooded slopes; occasional.

Hackelia virginiana (L.) I.M. Johnst. Wooded slopes; occasional.

**Lithospermum arvense* L. Roadsides; fields; common.

Lithospermum latifolium Michx. Wooded slopes; rare.

Mertensia virginica (L.) Pers. ex Link. Lowland woods; occasional.

Myosotis macrosperma Engelm. Lowland woods; occasional.

Myosotis verna Nutt. Fields; roadsides; wooded slopes; common.

Phrymaceae

Phryma leptostachya L. Lowland woods; occasional.

Verbenaceae

Phyla lanceolata (Michx.) Greene. Along streams; common.
Verbena bracteata Lag. & Rodr. Roadsides; occasional.
Verbena hastata L. Roadsides; occasional.
Verbena simplex Lehm. Roadsides; fields; occasional.
Verbena stricta Vent. Roadsides; fields; common.
Verbena urticifolia L. Roadsides; fields; edge of woods; common.

Labiatae

Blephilia ciliata (L.) Benth. Wooded slopes; occasional.
Blephilia hirsuta (Pursh) Benth. Wooded slopes; occasional.
Cunila origanoides (L.) Britt. All woodland types; particularly abundant in bluff top woods.
Hedeoma pulegioides (L.) Pers. Wooded slopes; occasional.
**Lamium amplexicaule* L. Waste ground; common.
**Lamium purpureum* L. Fields; roadsides; occasional.
Lycopus virginicus L. Along Bay Creek; occasional.
Monarda bradburiana Beck. Slope and bluff top woods; common.
Monarda fistulosa L. Slope and bluff top woods; common.
**Prunella vulgaris* L. Waste ground, particularly disturbed areas along streams; common. This species is highly variable in our area.
Pycnanthemum flexuosum (Walt.) BSP. All woodland types; common.
Pycnanthemum incanum (L.) Michx. Wooded slopes; rare.
Pycnanthemum pycnanthemoides (Leavenw.) Fern. Wooded slopes; occasional.
Salvia lyrata L. Flood-plain woods; occasional.
Scutellaria elliptica Muhl. Slope and bluff top woods; occasional.
Scutellaria incana Biehler. Wooded slopes; occasional.
Scutellaria leonardii Epling. Bluff top woods; occasional.
Scutellaria ovata Hill. Slope and bluff top woods; occasional.
Stachys tenuifolia Willd. Along streams; occasional.
Teucrium canadense L. Along streams; occasional.

Solanaceae

**Datura stramonium* L. Fields; occasional.
Physalis pubescens L. Roadsides; rare.
Physalis subglabrata Mack. & Bush. Roadsides; fields; occasional.
Physalis virginiana Mill. Roadsides; fields; occasional.
Solanum carolinense L. Waste ground; occasional.
**Solanum nigrum* L. Moist soil in woods, fields, and along roads; occasional.

Scrophulariaceae

Aureolaria flava (L.) Farwell. Slope and bluff top woods; occasional.
Gerardia tenuifolia Vahl. Edge of fields; along streams; occasional.
Mimulus alatus Ait. Along several streams; common.
Pedicularis canadensis L. Lowland and slope woods; occasional.
Penstemon calycosus Small. Wooded slopes; occasional.
Penstemon deamii Pennell. Bluff top woods; rare.
Penstemon digitalis Nutt. ex Sims. Lowland woods; occasional.
Penstemon pallidus Small. Slope and bluff top woods; common.
Scrophularia marilandica L. Lowland and slope woods; occasional.
**Verbascum thapsus* L. Fields; roadsides; occasional.
**Veronica arvensis* L. Fields; roadsides; common.
Veronica peregrina L. Fields; roadsides; common.
Veronicastrum virginicum (L.) Farwell. All woodland types; occasional.

Acanthaceae

Dianthera americana L. In and along all streams; common.
Ruellia humilis Nutt. Slope and bluff top woods; common.
Ruellia pedunculata Torr. ex Gray. Roadside; only one plant found.
Ruellai strepens L. Edge of woods; occasional.

Orobanchaceae

Epifagus virginiana (L.) Bart. Lowland woods; occasional.

Bignoniaceae

Bignonia capreolata L. Lowland woods; bluff tops; occasional.

Campsis radicans (L.) Seem. Floodplain woods; occasional.

Plantaginaceae

Plantago aristata Michx. Waste ground; common.
**Plantago lanceolata* L. Waste ground; common.
Plantago pusilla Nutt. Exposed sandstone bluff tops; occasional.
Plantago rugelii Decne. in DC. Waste ground; common.
Plantago virginica L. Waste ground; common.

Rubiaceae

Cephalanthus occidentalis L. Along some of the streams; occasional.
Diodia teres Walt. Fields; roadsides; bluff tops; common.
Galium aparine L. Woods; roadsides; common.
Galium circaezans Michx. Moist soil; occasional.
Galium concinnum Torr. & Gray. Along streams; wooded slopes; occasional.
Galium obtusum Bigel. Along streams; occasional.
Galium pilosum Ait. Slope and bluff top woods; occasional.
Houstonia lanceolata (Poir.) Britt. Wooded slopes; occasional.
Houstonia longifolia Gaertn. Exposed sandstone bluff top; rare.
Houstonia pusilla Schoepf. Exposed bluff tops; common.
Mitchella repens L. Moist, shaded soil and boulders; occasional.

Caprifoliaceae

Lonicera flavescens Small. Lowland woods; rare.
**Lonicera japonica* Thunb. Waste ground; common.
Sambucus canadensis L. All woodland types; fields; common.
Symphoricarpos orbiculatus Moench. All woodland types; common.
Triosteum angustifolium L. Bluff top woods; rare.
Viburnum dentatum L. Along Hunting Branch; rare.
Viburnum prunifolium L. Wooded slopes; occasional.
Viburnum rufidulum Raf. Lowland and slope woods; occasional.

Campanulaceae

Campanula americana L. Lowland and slope woods; common.
Triodanis perfoliata (L.) Nieuwl. Slope and bluff top woods; fields; roadsides; common.

Lobeliaceae

Lobelia cardinalis L. Along some streams; occasional.
Lobelia inflata L. Edge of woods; along streams; fields; roadsides; common.
Lobelia leptostachys A. DC. Wooded slopes; rare.
Lobelia spicata Lam. Slope and bluff top woods; occasional.

Valerianaceae

Valeriana pauciflora Michx. Lowland woods; occasional.
Valerianella radiata (L.) Dufr. Along streams; occasional.

Compositae

**Achillea lanulosa* Nutt. Bluff top woods; one station found.
**Achillea millefolium* L. Fields; roadsides; common.
**Ambrosia artemisiifolia* L. Waste ground; common.
**Ambrosia bidentata* Michx. Exposed bluff tops; occasional.
**Ambrosia trifida* L. Waste ground; occasional.
Antennaria plantaginifolia (L.) Hook. All woodland types; common.
Aster anomalus Engelm. Wooded slopes; rare.
Aster ericoides L. Fields; common.
Aster exiguus (Fern.) Rydb. Fields; occasional.
Aster laevis L. Along a few of the streams; occasional.
Aster laterifolius (L.) Britt. Along Spring Branch; one station found.
Aster novae-angliae L. Wooded slopes; occasional.
Aster ontarionis Wieg. Fields; occasional.
Aster patens Ait. Wooded slopes; occasional.
Aster pilosus Willd. Fields; common.
Aster praealtus Poir. Fields; occasional.
Aster sagittifolius Wedem. Wooded slopes; occasional.
Aster shortii Lindl. Wooded slopes; occasional.
Aster simplex Willd. Fields; occasional.
Aster vimineus Lam. Fields; occasional.
Bidens aristosa (Michx.) Britt. Along some of the streams; occasional.
Bidens bipinnata L. Lowland woods; along streams; common.
Cacalia atriplicifolia L. Wooded slopes; occasional.
Cacalia muhlenbergii (Sch.-Bip.) Fern. Lowland woods; rare.

Chrysanthemum leucanthemum L. Slope and bluff top woods; roadsides; fields; common.
Cirsium altissimum (L.) Spreng. Roadsides; occasional.
Cirsium discolor (Muhl.) Spreng. Bluff top woods; occasional.
**Cirsium vulgare* (Savi) Airy-Shaw. Roadsides; fields; occasional.
Coreopsis tripteris L. Lowland and slope woods; occasional.
Eclipta alba (L.) Hassk. Along streams; occasional.
Elephantopus carolinianus Willd. Lowland woods; occasional.
Erechtites hieracifolia (L.) Raf. Wooded slopes; occasional.
Erigeron annuus (L.) Pers. Edge of woods; fields; roadsides; common.
Erigeron canadensis L. Fields; roadsides; common.
Erigeron philadelphicus L. Wooded slopes and bluff tops; fields; occasional.
Erigeron pulchellus Michx. Wooded slopes; occasional.
Erigeron strigosus Muhl. Roadsides; fields; woodlands; common.
Eupatorium coelestinum L. Lowland woods; along streams; common.
Eupatorium purpureum L. Lowland woods; occasional.
Eupatorium rugosum Houtt. Lowland woods; common.
Eupatorium serotinum Michx. Fields; common.
Gnaphalium obtusifolium L. Wooded slopes; occasional.
.*Gnaphalium purpureum* L. Bluff top woods; occasional.
Helianthus decapetalus L. Wooded slopes; common.
Helianthus divaricatus L. Wooded slopes; common.
Helianthus grosseserratus Martens. Wooded slopes; occasional.
Helianthus hirsutus Raf. var. *trachyphyllus* Torr. Field; one plant found.
Helianthus strumosus L. Wooded slopes; occasional.
Hieracium gronovii L. Slope and bluff top woods; occasional.
Krigia biflora (Walt.) Blake. Wooded slopes; occasional.
Krigia dandelion (L.) Nutt. Bluff top woods; occasional.
Krigia oppositifolia Raf. Moist areas in fields; occasional.
Krigia virginica (L.) Willd. Wooded slopes: occasional.
Lactuca canadensis L. var. *latifolia* Kuntze. Fields; roadsides; occasional.
Liatris pycnostachya Michx. Bluff top woods; occasional.
Liatris scabra (Greene) K. Schum. Exposed sandstone bluff tops and in the woods; occasional.
Parthenium integrifolium L. Bluff top woods; occasional.
Polymnia canadensis L. Lowland woods; occasional.
Polymnia uvedalia L. Lowland woods, often among the boulders; rare.
Prenanthes altissima L. Moist, shaded sandstone cliffs; occasional.
Pyrrhopappus carolinianus (Walt.) DC. Roadsides; occasional.
Rudbeckia hirta L. Bluff top woods; fields; roadsides; common.
Rudbeckia subtomentosa Pursh. Wooded slopes; occasional.
Senecio aureus L. Along and in stream beds; common.
.*Senecio glabellus* Poir. Lowland woods; occasional.
Silphium integrifolium Michx. Roadside; one plant found.
Silphium perfoliatum L. Along Bay Creek; one colony found.
Solidago altissima L. Fields; occasional.
Solidago canadensis L. Fields; occasional.
Solidago caesia L. Lowland and slope woods; common.
Solidago juncea Ait. Roadsides; occasional.
Solidago missouriensis Nutt. Bluff top woods; rare.
Solidago nemoralis Ait. Fields; wooded slopes; common.
Solidago ulmifolia Muhl. Slope and bluff top woods; common.
**Taraxacum officinale* Weber. Waste ground; common.
Verbesina alternifolia (L.) Britt. Lowland and slope woods; occasional.
Verbesina helianthoides Michx. Floodplain woods along Bay Creek; bluff top woods; occasional.
Vernonia fasciculata Michx. Bluff top woods; occasional.
Vernonia missurica Raf. Fields; occasional.

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Table 1. — Quadrat data from 5 lowland woods stations (10 random pairs for each. Station 1, 43.0 trees per acre; Station 2, 88.1 trees per acre; Station 3, 186.8 trees per acre; Station 4, 124.9 trees per acre; Station 5, 37.1 trees per acre).

Species	% Dominance					Relative Density					% Frequency				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Acer saccharum	4.9	13.5	13.1	29.4	5.7	10	10	20	30	7	20	20	40	50	14
Betula nigra	8.9	....	....	5.7	....	5	..	..	10	..	10	..	..	20	..
Carpinus caroliniana	1.5	0.8	....	....	....	5	5	..	..	..	10	10	..	..	..
Carya glabra	....	1.6	....	....	....	..	5	..	..	..	..	10	..	..	..
Carya ovata	....	1.1	....	1.4	....	..	5	..	5	..	..	10	..	10	..
Carya sp.	....	....	4.6	....	5.8	..	..	15	..	14	..	..	20	..	28
Cornus florida	....	....	....	....	0.7	..	..	..	..	7	..	..	..	..	14
Fagus grandifolia	....	60.4	58.4	41.1	70.5	..	50	35	30	50	..	60	60	50	71
Fraxinus spp.	....	....	1.2	1.0	....	..	..	5	5	..	..	..	10	10	..
Liriodendron tulipifera	45.6	....	21.1	15.4	....	15	..	20	5	..	20	..	30	10	..
Nyssa sylvatica	....	9.2	....	....	9.4	..	5	..	..	8	..	10	..	..	14
Quercus alba	....	12.6	1.2	1.4	....	..	15	5	5	..	..	30	10	10	..
Quercus rubra	39.1	0.8	....	4.5	7.9	65	5	..	10	14	70	10	..	20	14

TABLE 2. — Quadrat data from 5 lowland woods stations (10 random pairs each).

Species	% Composition					% Frequency				
	1	2	3	4	5	1	2	3	4	5
Acer rubrum					1.4					10
Acer saccharum	1.0	5.4	5.3	0.5	4.3	10	80	60	10	10
Allium canadense	0.4					20				
Ambrosia trifida	7.0		0.4			30		10		
Arisaema triphyllum	1.0	6.7				10	50			
Arundinaria gigantea	0.5			11.4	6.5	10			30	30
Asarum reflexum	6.3					20				
Asimina triloba		0.4		1.5	0.3		10		10	10
Botrychium virginianum		0.5	0.5				10	10		
Brachyelytrum erectum		15.0					30			
Bromus ciliatus	0.6			0.4		20			10	
Carex spp	0.5	4.5	2.0	0.5	1.4	10	20	10	10	10
Carpinus caroliniana		0.3		1.0	3.6		10		10	30
Carya cordinformis	3.0					10				
Carya ovalis		3.5	1.5				10	10		
Carya ovata		0.5					10			
Cireaea latifolia	2.5					20				
Cornus florida		0.8					10			
Corylus americana					2.9					10
Cryptotaenia canadensis		3.3					10			
Danthonia spicata			9.5	2.0	10.0			20	20	30
Desmodium nudiflorum		2.5		1.0	0.7		30		10	10
Desmodium spp			3.0					10		
Dioscorea quaternata	0.1				3.6	10				20
Dryopteris hexagonoptera					4.3					10
Dryopteris marginalis		0.5					10			
Elephantopus carolinianus	8.0					70				
Epifagus virginiana		0.5			0.7		10			10
Euonymus atropurpureus			29.4	0.5				40	10	
Eupatorium spp	4.8			2.0		80			10	
Euphorbia corollata		0.5					10			
Fagus grandifolia		2.8				40				
Festuca spp	0.8					20				
Fraxinus spp		2.0	0.1	0.9			30	10	20	
Galium aparine	1.9	0.5				60	10			
Galium circaezans	0.7		0.1			20		10		
Galium concinnum	1.1	9.4		3.0		30	60		30	
Geum canadense	1.2					30				
Hybanthus concolor	0.6					20				
Hydrangea arborescens			2.5	2.5	10.0			10	10	30
Hydrophyllum spp	1.2					30				
Impatiens biflora	2.2	0.3		2.0		40	10		10	
Juniperus virginiana		0.7					20			
Lindera benzoin	14.0		1.1	0.5		70		20	10	
Luzula multiflora	0.1					10				
Nyssa sylvatica		0.2	1.5	2.5	8.6		10	10	20	30
Ostrya virginiana		1.0	2.0	8.0			20	10	20	
Oxalis stricta	0.5			0.5		10			10	
Panicum boscii	0.7					10				
Panicum latifolium	0.3	1.0				20	10			
Panicum microcarpon	0.5	0.5	18.0		0.8	10	10	40		10
Panicum polyanthes				2.0					20	

TABLE 2—(Continued)

Species	% Composition					% Frequency				
	1	2	3	4	5	1	2	3	4	5
Panicum spp.	0.3			0.5		10			10	
Parthenocissus quinquefolia	10.5	9.7	4.6	2.0	9.3	50	50	40	20	70
Passiflora lutea			0.1		0.2			10		10
Phlox divaricata	0.1					10				
Poa sylvestris	0.3					10				
Podophyllum peltatum			0.3					10		
Potentilla simplex	0.3		0.5			20		10		
Prunus serotina		0.5	1.5				10	10		
Quercus alba				1.4	2.9				20	10
Quercus rubra			0.5	0.5	0.9			10	10	30
Ranuuculus recurvatus	0.1					10				
Rhus radicans	2.7	4.7	20.5	15.5	15.0	20	30	40	40	50
Rudbeckia hirta	0.1					10				
Salvia lyrata	0.4					10				
Sanguinaria canadensis	0.5					10				
Sanicula spp.	0.4					20				
Sassafras albidum		1.0	2.0	2.0	0.8		20	20	10	10
Sedum ternatum	0.4					10				
Smilacina racemosa			0.5		2.9			10		10
Smilax bona-nox		0.2					10			
Smilax glauca			0.9	3.7				30	30	
Smilax hispida	1.4					40				
Smilax rotundifolia		5.5					10			
Solidago caesia	1.0		0.5			10		10		
Staphylea trifolia					3.0					10
Symphoricarpos orbiculatus	0.5			0.4		10			10	
Ulmus rubra	0.1	0.7	0.5	0.5		10	20	10	10	
Uniola latifolia	0.6	0.5	2.0			20	10	10		
Valeriana pauciflora	2.0					10				
Viola eriocarpa	0.3					10				
Viola papilionacea	1.6					50				
Viola sororia	2.0	0.8	0.5	5.0		60	20	10	30	
Viola striata	12.8					80				
Vitis spp.			5.0	0.3	4.5			10	10	20
Woodsia obtusa			0.1					10		

TABLE 3. — Quadrat data from 3 blufftop woodland stations. Station 1 (10 random pairs), 119 trees per acre, with 23.1 feet average distance between trees; Station 2 (6 random pairs), 110 trees per acre, with 24 feet average distance between trees; Station 3 (10 random pairs), 99 trees per acre, with 26.1 feet average distance between trees.

Species	% Dominance			Relative Density			% Frequency			Basal Area		
	1	2	3	1	2	3	1	2	3	1	2	3
Acer rubrum	....	....	2	....	....	5	....	....	10	.....	.....	28.3
Fraxinus americana	....	5	....	....	8	....	....	17	....	.....	28.3	.....
Juniperus virginiana	29	8	7	35	17	15	50	17	30	230.0	40.8	82.4
Quercus alba	32	49	11	15	8	15	20	17	20	251.9	254.3	126.3
Quercus marilandica	23	14	....	25	17	....	40	33	....	179.7	66.7	.....
Quercus stellata	13	19	78	20	42	60	30	67	80	103.6	101.3	934.2
Quercus velutina	3	....	....	5	....	....	10	....	....	28.2	.....	.....
Ulmus alata	....	5	....	....	8	....	....	17	....	.....	28.3	.....
Ulmus americana	....	....	2	....	....	5	....	....	10	.....	.....	28.3

TABLE 4. — Quadrat data from 3 blufftop woodland stations. Station 1, 10 random pairs; Station 2, 6 random pairs; Station 3, 10 random pairs.

Species	% Composition			% Frequency		
	1	2	3	1	2	3
Acalypha virginica	.....	0.1	.....	....	17	....
Agave virginica	.....	.....	0.3	....	....	10
Andropogon virginicus	0.5	15.5	24.0	10	33	50
Antennaria plantaginifolia	16.4	0.9	16.5	30	33	70
Carex spp.	.....	.....	0.2	....	....	10
Carya spp.	0.2	.....	0.1	10	....	10
Cheilanthes lanosa	.....	.....	0.2	....	....	10
Crotonopsis elliptica	0.3	2.1	0.6	20	67	30
Cunila origanoides	3.2	.....	1.0	40	....	40
Danthonia spicata	40.2	19.1	18.2	80	83	70
Desmodium spp.	.....	.....	1.5	....	....	20
Diodia teres	.....	1.7	.....	....	17	....
Elymus virginicus	.....	0.2	.....	....	17	....
Euphorbia corollata	0.1	.....	0.5	10	....	20
Festuca octoflora	2.5	3.3	.....	10	17	....
Galium circaezans	.....	.....	0.1	....	....	10
Helianthus divaricatus	0.4	.....	2.8	20	....	70
Hieracium gronovii	5.3	.....	0.2	30	....	10
Houstonia longifolia	0.1	.....	.....	10	....	....
Hypericum drummondii	.....	0.3	.....	....	17	....
Juncus tenuis	.....	8.7	.....	....	17	....
Juniperus virginiana	.....	0.1	0.1	....	17	20
Lechae tenuifolia	0.1	.....	.....	10	....	....
Lespedeza spp.	11.3	0.1	5.3	50	17	70
Opuntia rafinesquii	4.9	.....	.....	10	....	....
Panicum linearifolium	2.5	.....	.....	10	....	....
Panicum polyanthes	.....	.....	0.1	....	....	10
Panicum praecocius	5.0	.....	.....	10	....	....
Panicum xalapensis	.....	1.7	.....	....	17	....
Panicum spp.	0.4	2.5	3.1	10	50	80
Polygonatum biflorum	0.2	.....	.....	10	....	....
Pycranthemum flexuosum	.....	1.7	.....	....	17	....
Quercus marilandica	0.9	0.5	0.1	20	17	10
Quercus stellata	.....	1.5	.....	....	17	....
Rhus radicans	.....	.....	0.6	....	....	20
Rosa spp.	.....	.....	0.8	....	....	10
Rudbeckia hirta	.....	.....	0.1	....	....	10
Smilax glavca	4.1	.....	0.2	20	....	20
Stylosanthes biflora	0.3	.....	5.8	20	....	30
Tephrosia virginiana	.....	.....	1.5	....	....	10
Ulmus alata	.....	.....	0.2	....	....	20
Vaccinium arboreum	.....	.....	16.5	....	....	70
Vitis spp.	.....	.....	0.2	....	....	10

TABLE 5. — Quadrat data from 10 quadrats in a roadside field (4A) and 10 quadrats in an open field (4B). Average basal area for the roadside field was 13.0%, for the open field 11.5%.

Species	% Composition		% Dominance	
	4 A	4 B	4 A	4 B
Allium vineale	0.1	10	0.2	20
Andropogon virginicum	89.8	100	91.2	100
Carex hirsutella	0.2	10	0.7	20
Cassia fasciculata	0.4	40	1.3	90
Cercis canadensis	0.1	10	.....	.....
Crotonopsis elliptica	0.2	20	0.2	20
Desmodium spp.	1.3	60	0.9	50
Diodia teres	0.2	10	.....	.....
Diospyros virginiana	0.2	10	.....	.....
Galium spp.	0.2	10	0.2	10
Juncus tenuis	.....	.....	0.1	10
Juniperus virginiana	.....	.....	0.2	10
Lespedeza spp.	0.8	50	0.3	30
Panicum spp.	0.4	30	0.4	30
Parthenium integrifolium	0.6	30	0.6	40
Parthenocissus quinquefolia	0.5	20	0 2	10
Potentilla simplex	.....	.....	0.6	30
Rhus copallina	0.4	30	0.3	20
Rubus spp.	1.5	50	.....	.....
Sassafras albidum	0.3	20	1.1	60
Smilax glauca	0.6	50	1.2	80
Solidago spp.	1.1	50	0.1	10
Ulmus alata	0.3	20	.....	.....
Unidentified grasses	0.7	50	.....	.....
Unidentified herb	.....	.....	0.2	20

EXPERIMENTAL APPLICATIONS OF MULTIBAND PHOTOGRAPHY IN URBAN RESEARCH

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ABSTRACT. — Planning and research within the urban area are placing increasing demands on urban data which cannot be met by existing sources. As a result, considerable recent interest has been shown in remote sensors as a possible source for satisfying some urban data needs.

Three studies in experimental applications of one of these sensors, the multiband camera, to urban data problems are reported. The first discusses visual interpretation of housing quality from associated environmental characteristics. The second is concerned with the identification of road surfaces by spectral signature, a procedure which will form the basis of subsequent automated surface identification. The final experiment illustrates a method of automated data retrieval from a set of multiband photographs.

In recent years, increasing interest has been shown in remote sensors as a means for investigating a variety of phenomena. Remote sensors are instruments which record data about objects while located at some distance from those objects. They may be carried on the ground, on aircraft, or on satellites. The conventional camera which takes photographs of phenomena (people, things, etc.) is an example of a remote sensor.

One area in which remote sensors will play a critical role in the near future is in the analysis of urban phenomena. Scientists face an ever-widening gap between information regarding urban land use that is needed and that which is available using present data collection procedures. Three empirical studies are described. They provide a stimulus for more intensive research by indicating how important contributions may be made to both theory and planning within the urban area.

Experimental studies at Northwestern have been mainly concerned with the interpretation of the returns from one specific sensor, the multiband camera. Other available sensor returns have been neither as numerous nor of the quality of the multiband camera photographs.

In conventional aerial photography, one image is produced recording the radiation reflected from the imaged surface. The wavelengths of this radiation lie between 0.4 and 0.7 microns (the visible band of the electro-magnetic spectrum). In multispectral photography, several simultaneous images of the same set of objects are produced, each image recording returns for a separate part of the "visible" and "near visible" spectrum (the normal range of such a system is from 0.4 to 0.9 microns which includes the near infrared).

We may recognize two categories of data that can be extracted from imagery:

i) data relating to phenomena which are directly observable on the imagery.

ii) data relating to phenomena whose existence has to be inferred from the presence of other features on the imagery which are known to be consistently associated with these phenomena.

The extraction of these data can be undertaken using either visual or automated interpretation methods.

The compilation of inventories from direct visual interpretation has received considerable attention in the literature. Although most of this material is concerned with the use of conventional aerial photography, the same results can be obtained with multispectral photography. In some cases, the results may be improved, as specific phenomena may be more readily identified by their level of energy radiation within narrow bands of the electro-magnetic spectrum.

The acquisition of the second type of data, compilation of 'inventories by surrogate', has received less attention. It forms the focus of the first study, in which a method of evaluating housing quality from multispectral photographs is outlined. The second and third studies are concerned with the factors involved in automated interpretation in the urban area; the second illustrates a pattern recognition technique, while the third outlines a method of automated data extraction and graphic display.

The instrument used to obtain the imagery for the empirical work was an Itek nine-lens multiband camera.[1] This camera comprises nine six-inch Leighty High-Resolution lenses, each with a 21° square field of view. The nine shutters are synchronized to function simultaneously so that the same ground area is imaged in each case. Gelatin filters are employed with each lens to restrict the recorded returns to specified wavelength bands. Six of the exposures are on two rolls of 70mm. panchromatic film and the remaining three on one roll of 70mm. infrared film; the spectral range of the nine images is from 0.4 to 0.9 microns. The wavelength band associated with each of the nine frames is given in Table 1.

Visual Interpretation: The Evaluation of Housing Quality[2]

Determination of the spatial distribution of different grades of housing quality is of considerable importance in the field of urban planning and research. Where public health and welfare are concerned, the identification of areas where standards are low is particularly relevant. Current methods of evaluation are time-consuming, expensive, and subject to considerable error. If remote sensing devices can provide a major portion of the required information, and, in so doing, reduce overall expenditures, an important service will have been performed.

Table 1: Multiband Camera Spectral Bands.

Lens No.	Bandwidth (mu)
1	400-500
2	450-510
3	520-550
4	550-600
5	590-640
6	670-720
7	700-810
8	810-900
9	Full sensitivity range of IR film

The study recognized that housing quality cannot be directly measured from the air, since quality of housing depends not only on external features of the dwelling and immediate environment, but also on the internal condition of the structure. Nevertheless, it was hypothesized that these internal characteristics are consistently associated with other external criteria that may be imaged from a remote sensor. The verification of such a hypothesis would facilitate the development of a rapid and efficient survey method by which areas having the highest probability of possessing low quality housing could be readily identified.

Fifteen small areas in the Chicago region, each corresponding to one multiband sequence, were chosen for detailed study. Inferences were made about the housing quailty in each area on the basis of the following criteria: land crowding, non-residential land uses, private open space, hazards and nuisances associated with the transport system, public utilities, and the presence of basic community facilities.

Although most of the above features can be identified on conventional aerial photographs, it was often found that a given phenomenon could be distinguished much more readily on one band than on any other. More significantly, some factors, which were found to be consistent indicators of poor housing quality, were not readily identifiable on any plate within the visible spectrum, but stood out clearly in the bands of the photographic infrared. The more important of these are listed below:

1. *Private Landscaping.* In the better quality housing areas, landscaping in the form of shrubs, rock gardens, flower beds, etc., is common to almost every lot. The opposite tends to be true in the poor quality areas, although occasionally a lot supporting a single-family dwelling in an area of predominantly poorer quality multiple-unit structures will exhibit some landscaping. This information is more readily obtained from the photographic infrared. The infrared also helps to differentiate grass and dirt. Grass-covered front and back yards are found in good quality housing areas, while in poorer areas, dirt rather than grass is evident. Where grass does exist in poorer districts, it is not as healthy or well-conditioned as in the better quality housing areas, and tends to be patchy in appearance. In addition, the photographic infrared clearly reveals footpaths crossing both occupied and vacant lots in the low quality housing areas.

2. *Public Landscaping.* Parkways (strips between streets and sidewalks) are the property of the city. However, the property-owner is obligated to care for that segment of the parkway which fronts on his property. In the better areas, this strip is invariably grass-covered and well-kept, comparable to the condition of the owner's private lawn. In the poorer quality housing areas the parkway is usually a strip of dirt. A few small, widely-spaced trees are found on the better-kept of these blocks. Structures in these areas are frequently multiple-family, so there may be some question as to whether the landlords or tenants are remiss in maintaining the parkways.

3. *Vacant Lots.* In the areas of better quality housing vacant lots are well-tended. The converse is true in poor quality areas. This difference may be due to the stronger influence of factors such as neighborhood pride, pressure from neighbors, and enforcement of city by-laws in higher quality residential areas. The photographic infrared clearly differentiates between grass, weeds, and bushes; in addition, litter may be more readily identified on the infrared than on returns from the visible portion of the spectrum.

4. *Curbing.* In areas of better quality housing the curb line is unbroken on all blocks. In the poorer quality areas, the curb line is frequently broken, as a result of cars being parked on parkways or front yards, or has not kept pace with building. The photographic infrared imagery reveals this feature more readily and reliably than the visible imagery.

Study of the fifteen areas using multiband imagery has led to some tentative statements as to those features which are most commonly associated with the occurrence of poor quality housing. These were found to be:

i) the presence of litter, garbage, wrecked or derelict cars, and piles of lumber and rubbish throughout a neighborhood, on both occupied and vacant lots. In the study area this proved to be the best single indicator of low quality housing.

ii) a lack of landscaping in yards and parkways together with the presence of weeds on vacant lots.

iii) the number of vacant lots.

iv) the existence of non-residential

hazards and nuisances, primarily industrial plants and warehouses.

v) the extent of lot-crowding, i.e., the amount of open or play area that exists on the residential lots.

In exploring the validity of the above technique it is instructive to compare the evaluation procedure with that used by the American Public Health Association (APHA).[3] Of the 24 items contained in the APHA environmental survey appraisal form, 20 can be estimated for small areas using multiband photographs. In addition, 10 items not included in the APHA survey form were found to be consistent indicators of low quality housing in the Chicago multiband study.

It is possible that the Bureau of the Census will rely heavily on the APHA appraisal form for providing environmental data in the 1970 or 1975 Census. If the form is adopted, the multiband technique will gain added significance since it could provide data on rapidly changing housing patterns that would be compatible with data obtained on a regular basis by the Census. The Census data would also provide ground truth which could be used to check for systematic errors in interpretation.

The extent to which multispectral photographs can replace the field enumerator has yet to be established by controlled experiment. A point to be made is that the socioeconomic background and experience of the enumerator is an important source of error in existing data; the employment of experienced photointerpreters would help to greatly reduce this variation, not only because of superior training but also because fewer persons are involved in data acquisition.

A. The Classification of Road Surfaces[4]

Urban transportation provides an exciting field for the potential application of remote sensing. Possibilities for use of both active and passive sensors range from continual updating of maps of the transportation network to inventories of rolling stock and the monitoring of traffic flows in real time. The utility of any one sensor depends on its particular characteristics; in the case of multispectral photography, the ability to distinguish between different surfaces on the basis of their patterns of spectral reflectivity suggests that the classification of road surfacing materials might prove a profitable avenue of research. This could possibly form the first step toward a system which not only provides an inventory of surfacing characteristics for a city but also indicates the state of repair of that surface, pinpointing those areas in need of attention.

Sets of photographs for the North suburban areas of Evanston, Glenview, and Wilmette were obtained from a flight over Chicago in 1965. These are compared with maps compiled from a concurrent ground survey.

Since unpaved and paved roads can be readily distinguished on conventional photographs, attention was focussed on the latter category. Four different surface types were recognized in the ground survey:

1. bituminous asphalt
2. asphaltic concrete
3. new concrete
4. old concrete

asphalt concrete

Visual inspection of the prints indicated that certain bands were more useful for distinguishing between these surfaces than others. For example, the greatest contrast between asphalt and concrete was to be found in the 0.55-0.60 micron range. Although the simultaneous visual comparison of several images is difficult, it did appear that the differences between band 4 (in the visible spectrum) and band 7 (in the near infrared) might provide a basis for discriminating between asphaltic concrete and bituminous asphalt. The evidence was sufficiently encouraging to justify further investigation.

Thirty-eight sample points on the images were chosen to cover the full range of surface types within the study area. Digitized values for these points were obtained by visual comparison with a Kodak Gray Scale on five of the nine available bands (Nos. 1, 2, 4, 7, and 8). Thus for the ith point we may define a vector X_i, where

$$X_i = (x_{i1}, x_{i2}, x_{i3}, x_{i4}, x_{i5}) \quad i = 1, 2, \ldots, 38$$

The x_{ij} are the individual tonal values for the ith point.

How may we determine the organization existing in this data set? One method is to consider X_i to define a point in five-space (with coordinates x_{i1}, x_{i2}, ..., x_{i5}). The distance from the point defined by X_i and that defined by X_5 ($=d_{ij}$) may then be defined as

$$d_{ij} = \sum_{k=1}^{5} (x_{ik} - x_{jk})^2 \; ^{\frac{1}{2}}$$

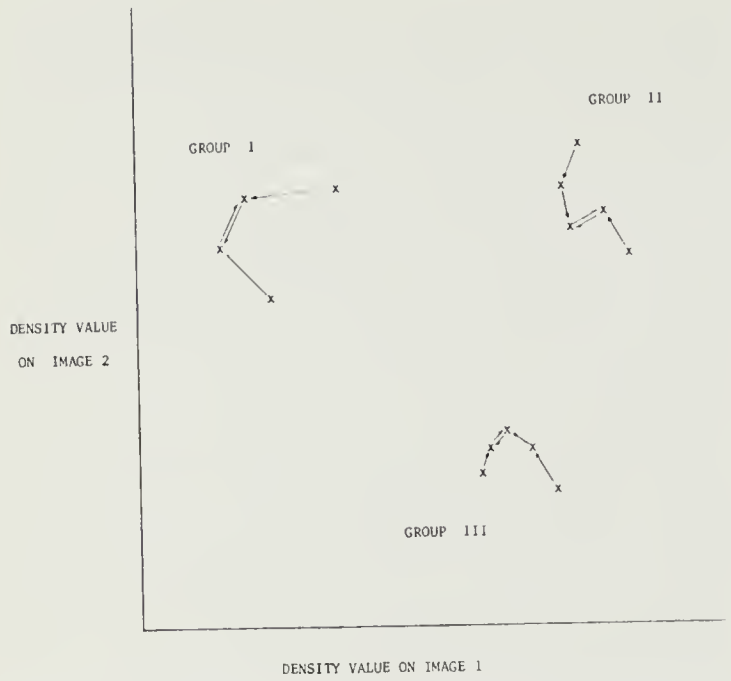

FIGURE 1.—Elementary linkage analysis in two-space.

Computation of the distances between all pairs of points yields a symmetrical matrix (with 38x38 elements in this case). Each of the distances indicates the "closeness" of two points in five-space; this would seem to be an important measure as we are concerned with the way in which the points form groups or clusters.

A simple way of subdividing the points into groups is to perform an elementary linkage analysis[5] on the distance matrix, such that each point is connected to its nearest neighbor. Figure 1 illustrates how such groups may be formed in two-space. In this way the basic organization of the data is identified; real world surfaces which are associated with these groups may then be established. Table 2 gives the results of this operation for the sample set of road surfaces.

The grouping procedure reveals important underlying patterns of associations between the signatures:

1. Bituminous asphalt is clearly separated from all other surfaces.
2. New and old concrete as a single category is well differentiated from the others; however, even within this classification some differentiation between old and new concrete may be possible. Group VI contains no new concrete, while the largest group, II, contains only two old concrete surfaces out of eleven.

TABLE 2.—Organization of Road Surface Signatures.

Group No.	Surface Code No.	Surface Type	Group No.	Surface Code No.	Surface Type
I	1*	asphaltic concrete		5	bituminous asphalt
	12	old concrete		28	bituminous asphalt
			V	34	bituminous asphalt
	2	new concrete		35	bituminous asphalt
	8	new concrete		41	bituminous asphalt
	4	new concrete			
	14	new concrete		6	old concrete
	19	new concrete		7	old concrete
	23	new concrete	VI	10*	asphaltic concrete
II	24	new concrete		11	old concrete
	29	asphaltic concrete		13	old concrete
	30	new concrete			
	33	new concrete	VII	16	bituminous asphalt
	36	old concrete		21	bituminous asphalt
	39	old concrete			
				18	old concrete
	3	asphaltic concrete		27	new concrete
III	25	asphaltic concrete		31	new concrete
			VIII	32	new concrete
	17	asphaltic concrete		37	old concrete
IV	22*	bituminous asphalt		38	old concrete
	26	asphaltic concrete		40*	asphaltic concrete

FIGURE 2. — Relationships between road surface groups.

3. All the main inconsistencies in the grouping (marked with an asterisk) involve the category "asphaltic concrete." Since some difficulty was encountered in trying to classify this surface in the ground survey, problems at a subsequent stage were not altogether unexpected.
4. Despite the difficulties with asphal-

FIGURE 3. — Residential subdivision -0.45 -0.51 mu.

FIGURE 4. — Retrieved pattern of large field.

tic concrete, the results are encouraging. When more data become available, it is hoped to extend this research, applying more rigorous techniques of analysis. With a large controlled sample, it should be possible to determine the parameters of the point distributions in n-space (n being the number of elements in each signature), i.e., the centroid and standard distance of each group in I can be computed. Given this information, it should then be possible to allot a given vector X_i to a similarity set S_j using a Bayesian decision rule, such that an optimal classification of signatures is obtained.

B. Retrieval from Digitized Imagery[6]

Three multiband photographs, each of the same ground area, were selected from a flight over Phoenix, Arizona. One was sensitive to radiation within the range 0.45-0.51 microns, one to radiation within the range 0.52-0.51 microns, and the third within the infrared range 0.80-0.90 microns. These are called images 1, 2, and 3. Image 1 is reproduced as Figure 3.

Each image was divided into 6400 cells (80x80). For each of these cells an average gray scale density was determined, this density being measured along a digital scale from zero to 999. In analyzing these data, a special program was used which provided for the specification of an allowable density range for a variable number of band widths.[7] For example, a rule may be set up such that if the density values for a given element lie between x_1 and x_2 for image 1, between y_1 and y_2 for image 2, and between z_1 and z_2 for image 3, then that element may be classified as a phenomenon P. The elements of the three images are searched sequentially,

and each cell that falls within all three limits is "retrieved"—in the cases cited below this takes the form of a locational plot (X) (for an example, see Figure 4).

Figure 4 represents an attempt to retrieve the elements of the large field shown in Figure 3. In order to obtain the density limits for each image, the mean and standard deviation of the density value for all elements of the field were computed for each image. If μ_1 and σ_1 are the mean and standard deviation respectively for the field elements in image 1 then the lower limit x_1 is set to

$$x_1 = \mu_1 - \sigma_1$$

and the upper limit x_2 is set to

$$x_2 = \mu_1 + \sigma_1.$$

y_1, y_2, z_1, and z_2 may be similarly defined.

Elements satisfying all three sets of limits are retrieved and plotted. Of the 2371 cells making up the field, 1164 were retrieved, together with 132 which satisfied the test but were not included within the field.

Figure 1 illustrates a similar experiment undertaken for road surfaces. Once again, the general real world pattern can be identified, but a considerable amount of extraneous data are also recovered.

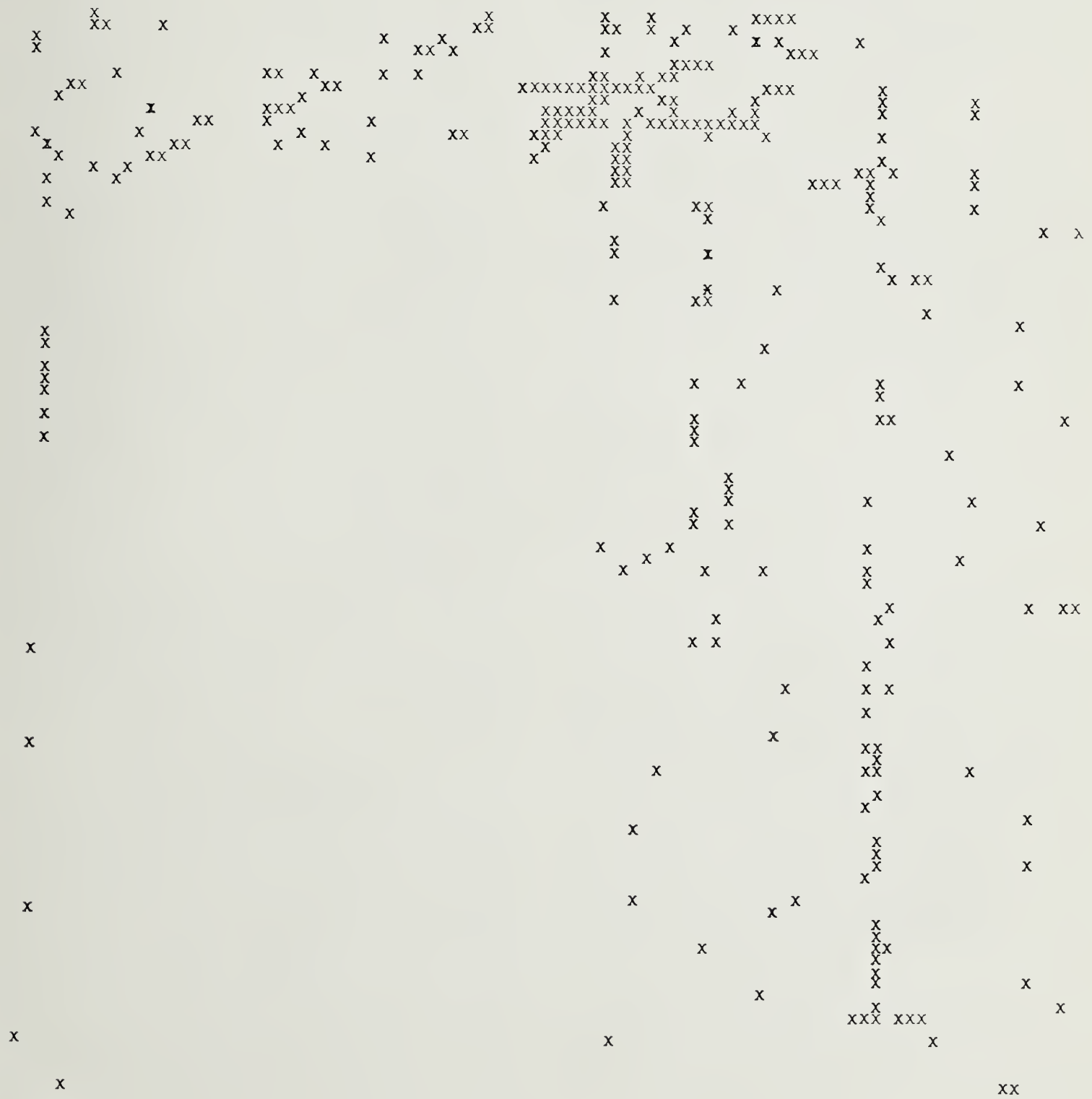

FIGURE 5. — Retrieved pattern of road surface.

The presence of these extraneous data in the two experiments is due to the relatively crude classification algorithms that were used. Surfaces outside the fields such as lawns and parkways were plotted in one case, and non-street surfaces such as concrete paths and patios were plotted in the other. This results from the nature of the recognition technique used, which simply plotted all elements of similar spectral signature in the respective experiments.

The main purpose of this exercise is to illustrate the type of output formats that can be utilized; as more sophisticated pattern recognition procedures are employed, elements satisfying different sets of conditions can be retrieved in the same run, and direct print-outs on that basis of a multiple classification can be achieved.[8]

Conclusion

This paper attempts to illustrate some of the encouraging results that have been obtained in using multispectral photographs to investigate topics of relevance to urban planning and research. It is stressed, however, that multiband imagery is but one type of remote sensing capability. Different sensors perform different functions; as technology advances and provides us with better imagery outside the visible range of the electro-magnetic spectrum (non-classified ultraviolet and thermal infrared imagery is not yet comparable to visible and near infrared photography in resolution capability) our ability to recognize and interpret will rapidly improve. The potential value of remote sensors in the urban area is not in question; the major task is to utilize this potential in the most efficient way.

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Acknowledgment

Support for this research was provided by the Earth Resources Survey Program, National Aeronautics and Space Administration, Contract No. 14-08-0001-10654.

Manuscript received May 5, 1967.

NEWS AND COMMENTS

The new editor after much toil, consultation with the former editor and with the assistance of the associate editor has his first issue of the *Transactions* ready for the printer. We look forward with some trepidation to seeing it in print. We have nightmares of inverted photographs, editorial errors and all the countless things that could be "fouled" up. The former editor assures us not to lose our cool.

In working over the many manuscripts and in looking over the comments of the reviewers who have done Trojan service, one item appears with great tenacity and frequency. The editors and the reviewers are almost unanimous in their opinion that the greatest sin committed by all of the authors is the sin of verbosity. All too frequently the comment is: The paper is too wordy; the manuscript should be greatly condensed. The rising cost of publication in other journals has caused this complaint to become a clamor. However, a somewhat more leisurely style of writing has been employed in the *Transactions* and probably legitimately so. But subsequently a somewhat more jaundiced view of this defect can be expected.

In retrospect two or three other consistent faults crop up. The first of these is the failure to follow the publication format formulated for authors who submit papers to the *Transactions*. The second is the failure to provide the editor with two copies of the manuscript. Specific instructions appear on the inside back cover.

The many authors and members of the academy may be yet unaware of the changed editorship. Dr. Galbreath after several years as editor, in which the *Transactions* under his direction has flourished and met its publication deadlines, has resigned. We salute him for a job well done. The new editor and associate editor are, respectively, Darrel L. Lynch and Wendell Lindbeck of the Biological Sciences and Chemistry departments at Northern Illinois University at DeKalb. The new team hopes to maintain the efficient pace set by Dr. Galbreath.

The Biology Department at Northern Illinois University has a new electron microscope. Dr. Knut Norstag, who has had training in electron microscopy, is in charge of the instrument.

The deadline for submitting requests to the Research Grants Committee is April 1. A copy of guidelines for preparing research grant proposals can be obtained from:

David G. Rands, Chairman
Research Grants Committee, ISAS
Southern Illinois University
Edwardsville, Illinois 62025

New publications of the Illinois State Museum, off the press in the

summer and fall of 1967, are:

Popular Science Series

Vol. VII WILD ORCHIDS OF ILLINOIS. Glen S. Winterringer. 130 p. 36 line drawings. 41 photographs. Paper Covers. $2.50.

Vol. VIII FRESH-WATER MUSSELS OF ILLINOIS. By Paul W. Parmalee. 108 p. 4 line drawings. 35 plates. Paper Covers. $2.50.

Reports of Investigations

No. 10 AN ARCHAEOLOGICAL SURVEY OF THE WABASH VALLEY. By Howard D. Winters. 95p. 23 line drawings. Paper Covers. $2.50.

No. 11 THE PALEOPATHOLOGY OF THE ARCHAIC MODOC ROCK SHELTER INHABITANTS. By Holm Wolfram Neuman. 68p. 33 plates. Paper Covers. $2.50.

No. 12 THE GENTLEMAN FARM SITE, LA SALLE COUNTY, ILLINOIS. By James A. Brown. 48p. 10 line drawings. 4 plates. Paper Covers. $2.50.

These publications are available from the Illinois State Museum Society, Illinois State Museum, Springfield, Illinois 62706. The above prices are subject to 4% sales tax for Illinois residents.

NOTES

NANNIZZIA INCURVATA, STOCKDALE ISOLATED FROM SOIL SAMPLES

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ABSTRACT.—Seven of 30 soil samples collected from two sites in Urbana, Illinois, were induced to produce the perfect form of *Microsporum gypseum*, which was identified as *Nannizzia incurvata*, Stockdale, 1961. Best results were achieved by using unsterile soil treated with an antibiotic solution (20 units of penicillin, 40 units of streptomycin and 0.5 mg of cycloheximide per ml) and baited with either child's hairs or horse hairs at 25°C. Cleistothecial formation was visible beginning from 20 to 25 days after soil samples were baited with hair.

Nannizzi (1927) was first to observe the perfect state of *Microsporum gypseum* to which he gave the name *Gymnoascus gypseum*. However, his work came under severe criticism because the substrates used were not sterilized and the cultures were, therefore, impure. Subsequent workers assumed that Nannizzi was mistaken and as a result, very little progress was made for more than three decades, until Dawson and Gentles (1959) using the hair bait technique, reported the perfect state of *Keratinomyces ajelloi*. Soon thereafter, Griffin (1960), Szathmary and Herpay (1960), and Stockdale (1961) independently discovered perfect states of *M. gypseum*. This discovery was confirmed in the following years in India (Sarkar, 1962; Mohapatra and Gugnani, 1964) and in Argentina (Varsavsky, 1964).

The discoveries of sexual states in other species of dermatophytes and related keratinophilic fungi followed in quick succession. Dawson and Gentles (1961) described the perfect state of *Microsporum nanum* and *Trichophyton terrestre*, Georg *et al.* (1962) of *M. vanbreuseghemii*, Ajello (1961) of *M. cookei*, Randhawa and Sandhu (1964) of *Keratinophyton terreum*, Stockdale *et al.* (1965) of *Trichophyton simii*, and Ajello and Cheng (1967) of *Trichophyton mentagrophytes*.

This information necessitated the reclassification of these fungi. Stockdale (1961), therefore, created a new genus *Nannizzia* to accommodate the perfect form of *M. gypseum*. She later classified the perfect state of *M. gypseum* in three different species of *Nannizzia*, namely, *N. incurvata*, *N. gypsea* and *N. fulva*. She included the perfect states of all species of *Microsporum* under the genus *Nannizzia* whereas those of *K. ajelloi* and *T. terrestre* were transferred to the genus *Arthroderma*. Comparatively recently, Dawson *et al.* (1964) studied the environmental conditions affecting sexual reproduction in the species belonging to these two genera.

The present work records for the first time the occurrence of *N. incurvata* in the United States. Various substrates, baits and growth temperatures were investigated to determine those most favorable for the growth of the perfect state.

MATERIALS AND METHODS

Thirty samples of soil were cultured for keratinophilic fungi: 14 were col-

lected from a garden bed and 16 from a ploughed field in Urbana, Illinois. Soil was scraped from surface layers of the two sites. After bringing the samples to the laboratory, they were poured in sterile petri plates; the plates were half filled. Each soil sample was moistened with aqueous antibiotic solution containing 20 units of penicillin, 40 units of streptomycin and 0.5 mg cycloheximide (Actidione, The Upjohn Co.) per ml and baited wtih either autoclaved human (child's) hair, horse hair, dog hair or chicken feathers by pressing tufts of hair or feathers partially into the soil. The plates were kept at 25° C. and examined at regular intervals.

In another set of experiments, three hair samples (1496, G3120, C2791) positive for *M. gypseum* were cultured separately on Sabouraud's dextrose agar. When luxuriant growth was obtained, an aqueous spore suspension was prepared by adding about 5 ml of sterile 0.85 per cent sodium chloride to each culture tube (25 x 150 mm) and admixing the culture and water thoroughly. About 2 ml of the suspension was inoculated in 20 petri plates which were half filled with sterile soil and baited with sterile child hairs, horse hairs, dog hairs, or chicken feathers. The plates were kept at room temperature (25° C.) and examined for 3 months at weekly intervals.

Results and Discussion

Of the 30 soil samples examined, seven yielded keratinophilic fungi that developed the perfect state; three from the garden bed and four from the ploughed field.

After 20 to 25 days of incubation at 25° C., cleistothecial formation was visible as small white tufts of hyphae which gradually enlarged, became pale-buff and finally yellowish-buff.

Cleistothecia were globose, pale to yellow-buff, measuring 255 to 500μ in diameter, exclusive of appendages. Peridial hyphae were numerous, septate, verticillately branched, thick-walled and with constrictions. Free ends were numerous; they were straight tapering hyphae which were slender, smooth walled and septate or they were hyphae which were slendor, smooth walled, septate and having loosely or tightly coiled spirals. Elliptical, rough walled macroconidia typical of *M. gypseum* were present.

On crushing the cleistothecium, the peridial hyphae collapsed and asci were released. The asci were globose to ovate in shape and measured 5 to 6 μ in diameter. Each ascus contained 5 to 8 ascospores.

The ascospores were light yellow, smooth walled and measured 2 to 3 μ by 1.5 to 2.0 μ.

In studies to determine the factors affecting the formation of perfect forms on hair, it was observed that cleistothecia were formed at 25° C. but not at 22° C. Child hair and horse hair but not dog hair or chicken feathers appeared to be most suitable for perfect stage formation. The perfect forms were not produced on sterile soil but were formed on unsterile soil treated with antibiotic solution.

None of the hair samples in the second experiment produced the perfect state though all of them had the characteristic growth of *M. gypseum*, forming cinnamon-brown colonies with numerous and characteristic macroconidia and microconidia.

Stockdale (1961) proposed the genus *Nannizzia* for (*N. incurvata*) the perfect state of *M. gypseum*. She later (1963) found certain differences between *N. incurvata* and *Gymnoascus gypseum* originally described by Nannizzi (1927) and came to the conclusion that they represented two separate but closely related species. She (*loc. cit.*) therefore proposed the name *Nannizzia gypsea* for Nannizzi's form. Morphologic characters of cleistothecia encountered in the present study agreed with the description of *Nannizzia incurvata*, Stockdale, 1961, except that in very few cases asci were seen having five instead of eight ascospores. The present form can be differentiated from *N. gypsea* and *N. fulva* (closely related species of *Nannizzia*) in that the peridial hyphae curved away from the cleistothecium whereas in the latter they curve towards it. Moreover, the presence of straight hyphal appendages was a constant feature of the cleistothecia of *N. incurvata* which occurs less frequently in the other two species.

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Manuscript received August 11, 1967.

SOME RESUPINATE BASIDIOMYCETES FOUND IN PENNSYLVANIA

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ABSTRACT. — Eleven species of resupinate Basidiomycetes are reported for Pennsylvania for the first time.

Recent collections of fungi previously unreported for Pennsylvania are noted here. The collections were made by the author (D.A.D.) on July 2nd, 1967 from a mixed hardwood forest with a good litter of decaying, deciduous wood. The collection site is located five miles west of Elliotsville, Pennsylvania in Fayette County. All the collections are deposited in the mycological herbarium of Illinois State University (ISU).

Peniophora cremea (Bres.) Sacc. & Syd.

On decayed deciduous wood. Very common, but previously unreported for Pennsylvania. Also known from New York, Ohio, New Jersey, West Virginia, Virginia, Illinois, Alabama, Michigan, Louisiana, Massachusetts, Vermont. VII. 2. 1967. (D.A.D.) 1105, 1106, 1108, 1109, 1111, 1112, 1138, 1143, 1144, 1145, 1070, 1077. (All at ISU).

Peniophora angusta Rog. & Weres.

On decayed deciduous wood. Previously unreported for Pennsylvania. Also known from Ontaria, British Columbia, Oregon, Rhode Island, and Massachusetts. VII. 2. 1967. (D.A.D.) 1116, 1104, 1069. (All at ISU).

Peniophora aspera (Pers.) Sacc.

On decayed deciduous wood. Previously unreported for Pennsylvania. Also known from Illinois, Australia, Denmark, Ukraine, Virginia, and New York. VII. 2. 1967. (D.A.D.) 1115, 1076, 1062. (All at ISU).

Peniophora subalutacea (Karst) Hoehn & Litsch.

On decayed deciduous wood. Previously unreported for Pennsylvania. Also know from England, France, Finland, Denmark, Poland, New Zealand, Sweden, Austria, British Columbia, Ontario, Illinois, Idaho, Louisiana, Alabama, New York, New Jersey, Maryland, and Washington. VII. 2. 1967. (D.A.D.) 1071. (At ISU).

Peniophora gracillima (Ellis & Everh. ex Rogers & Jackson) G. H. Cunn.

On decayed deciduous wood. Common, but previously unreported for Pennsylvania. Also known from South Africa, Manitoba, New Zealand, Italy, Austria, Australia, England, British Columbia, Ontario, Quebec, Sweden, New York, New Jersey, Vermont, Illinois, New Hampshire, Oregon, and Washington. VII. 2. 1967. (D.A.D.) 1103, 1129, 1130, 1131, 1134. (All at ISU).

Peniophora tenuis (Pat.) Massee

On decayed deciduous wood. Previously unreported for Pennsylvania. Also know from Ontario, Austria, Bermuda, France, Germany, Finland, Denmark, Poland, South Africa, Jamaica, Sweden, New Hampshire, New Jersey, New York, Massachusetts, District of Columbia, Illinois, Idaho, Montana, Oregon, and Washington. VII. 2. 1967. (D.A.D.) 1132, 1133. (Both at ISU).

Peniophora pubera (Fries) Sacc.

On decayed deciduous wood. Previously unreported for Pennsylvania. Also known from Australia, Austria, Canada, Denmark, France, Germany, Great Britain, Italy, Sweden, Alabama, District of Columbia, Florida, Idaho, Illinois, Kentucky, Louisiana, Maryland, Massachusetts, Missouri, Montana, New Hampshire, New Jersey, New York, Oregon, Rhode Island, Virginia, Washington, and Wisconsin. VII. 2. 1967. (D.A.D.) 1078. (At ISU).

Peniophora burtii Romell

On decayed deciduous wood. Previously unreported for Pennsylvania. Also known from New York, New Jersey, Alabama, Louisiana, Ohio, Vermont, West Virginia, Virginia, Illinois, Montana, Massachusetts, and Michigan. VII. 2. 1967. (D.A.D.) 1135, 1136. (Both at ISU).

Sistotrema brinkmanni (Bres.) Eriksss.

On decayed deciduous wood. Previously unreported for Pennsylvania. Also known from Austria, England, British Guiana, Panama, Nova Scotia, Ontario, Massachusetts, Rhode Island, New York, Virginia, North Carolina, Ohio, Tennessee, Louisiana, Iowa, Idaho, Missouri, and Oregon. VII. 2. 1967. (D.A.D.) 1110, 1068. (Both at ISU).

Corticium furfuraceum Bres.

On decayed deciduous wood. Previously unreported for Pennsylvania. Also known from British Columbia, Ontario, Idaho, North Carolina, and Wisconsin. VII. 2. 1967. (DA.D.) 1064, 1065. (Both at ISU).

Trechispora farinacea (Pers. ex Fr.) Liberta

On decayed deciduous wood. Reported for Wisconsin and Iowa. Common throughout the eastern United States. VII. 2. 1967. (D.A.D.) 1114, 1080. (Both at ISU).

Summary

Eleven new reports of resupinate Basidiomycetes are noted here for the first time. Although these notes represent no major range extensions, they aid in filling existing distribution gaps in the eastern United States.

Acknowledgments

The author wishes to express his thanks to Dr. Anthony E. Liberta and Mr. Robert J. Harris for their ready assistance in confirming and discussing some of the identifications. A special note of thanks is extended to Mr. Michael DeFigio for his help in the collection of these specimens.

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Manuscript received August 9, 1967.

AN AMOEBA ISOLATED FROM THE LATEX OF *EUPHORBIA MARGINATA* PURSH. (EUPHORBIACEAE)

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ABSTRACT. — *Hartmannella castellanii* Douglas was isolated from the latex of subterranean portions of *Euphorbia marginata* Pursh. (Euphorbiaceae). Eighteen of 161 plants from Illinois and Minnesota harbored the amoeba. This organism, which has recently become famous as a contaminant and pathogen, is reported for the first time from a plant. Methods for obtaining bacteria-free amoebae and culturing them axenically are presented. A generation time of 25.5 hours was obtained. A description of the vegetative and cyst stages is given.

During a search for endophytic microorganisms of laticiferous plants, *Hartmannella castellanii* Douglas, an amoeba, and a bacillus resembling *Bacillus megaterium* DeBary were found in the latex of *Euphorbia marginata* Pursh. (Euphorbiaceae). Information concerning the latex bacterium has been published (Troll, 1966). Amoebae were reported previously from latex and nonlatex plants.

Musgrave and Glegg (1904) described an amoeba found in the latex of *Lactuca* sp. These authors cultivated this organism in very simple nutrient broth (water and beef extract) and on solid medium. Schoevers (1918) recognized amoebae in the roots of diseased spinach plants. McWhorter (1922) isolated an amoeba, having many characteristics of *Entamoeba*, from cells of Fiji galls of sugar cane, *Saccharum officinarum* L. (Graminae). He proposed the name *Phytamoeba sacchari* for the organism. It can be cultured in cane juice.

Franchini, in his series of works on the protozoa of plants (1922-1923) has described some amoebae parasitizing a large number of laticiferous plants, most of them tropical, growing in botanical gardens either in greenhouses or out of doors. Three of these amoebae were named *Amoeba chlorocodonis*, *A. cryptostegiae*, and *A. lactucae*. Franchini's works on the amoebae of plants including a list of all host plants is summarized by Nieschultz (1925) and Wenyon (1926).

Bruni (1925) found some protozoa of amoeboid nature in the latex of *Acokanthera venenata* (Apocynaceae). His diagrams certainly appear like amoebae but detail is lacking.

DuPorte (1925) describes some amoeboid protozoa from the latex of *Convolvulus sepium* L. (Convolvulaceae). No reports of amoebae in latex plants have appeared in the literature since the above mentioned paper.

MATERIALS AND METHODS

Latex occurs in the plant families Apocynaceae, Asclepiadaceae, Compositae, Euphorbiaceae, Moraceae, Papaveraceae, and Urticaceae. Latex samples were collected in Alabama, Florida, Illinois, Indiana, Iowa, Minnesota, Missouri, Tennessee, and Wisconsin. A minimum of 5 samples from different portions of each plant including subterranean structures were examined. Since microorganisms tend to abound at the surface of the plant, sterile techniques were used in extracting the latex.

A solution consisting of 0.25 gm mercuric chloride, 0.25 gm phenol, 1.25 ml concentrated HC1 in 70% alcohol was used as a sterilizing agent. Sharp scalpels were kept in this solution for a minimum of 10 minutes and rinsed in sterile distilled water immediately prior to use. The plant parts were first

cleaned with water to remove the larger particles of dirt, etc., then the parts to be cut were swabbed with Lugol's iodine solution using a sterile cotton swab and allowed to dry. After the cut, the exuding latex was dropped onto a clean miscroscope slide. A thick smear was made with a sterile platinum loop which had been flamed using a cigarette lighter. After the smear was made, the loop was streaked over a nutrient agar slant. The smear was allowed to dry and was immersed in Schaudinn's fluid for 10 minutes. It was then rinsed with water and allowed to dry. The slides were marked with a notation indicating species and locality. Upon returning to the laboratory all smears were stained for 20 seconds with crystal violet and excess stain was removed with tap water. The slides were then dried using bibulous paper and thus were ready for microscopic observation. The use of this simple bacterial stain made it possible to stain many smears in a short time and those from infected plants could easily be distinguished at 430X magnification.

Nutrient agar slants that had been inoculated in the field were incubated for 48 hours at 25° C. and any growth on them examined under the microscope.

In order to isolate the amoebae from bacteria present, a sample of latex was taken aseptically, mixed with sterile saline solution, poured in a thin film on a nutrient agar plate, and incubated for 24 hours at 25° C. The superficial film of the plate was washed with sterile saline into a sterile centrifuge tube. Amoebic cysts were almost freed from bacteria by repeated centrifugation (five times) and resuspension in sterile saline. The sediment of the final centrifugation was streaked on a nutrient agar plate and incubated for 24 hours at 25° C.

For cultivation of the amoebae a liver-peptone-glucose (LPG) medium was prepared. The ingredients of the liquid phase are as follows: 50 g Bacto liver (Difco), 10g proteose peptone (Difco), 5g peptonized milk (Difco), 10g glucose, 2g NaCl, 0.2 Na_2HPO_4, 0.2g KH_2PO_4, 0.003g $MgCl_2 \cdot 6H_2O$, 0.003g $CaCl_2$, 0.003g $FeSO_4$, and 1000ml distilled water. To prepare the solid phase 1.5% agar is added to the broth and slants prepared after autoclaving. Each slant is overlaid with 2ml of sterile nutrient broth. The medium was inoculated with one tenth ml of amoebic cyst suspension. Growth curves were determined by inoculating one half ml of washed 72 hour original culture into each of 10 tubes of fresh medium. The tubes were incubated at 25° C. Every 24 hours, up to 12 days, 0.1ml samples were withdrawn and pooled. Numbers of cells were determined by direct counts using the 'Improved Neubauer' type of hemocytometer.

Sterility tests for the detection of bacterial and fungal contaminants were carried out for all cultures using the media and techniques of Diamond (1961).

Most observations were made on living amoebae using the phase contrast microscope. Janus green B in absolute ethanol (1:10,000) was used to stain mitochondria and the standard Feulgen nucleal reaction was performed.

Results

The amoebae were found only in the latex of subterranean parts of *Euphorbia marginata*. Eighteen of 161 plants from Illinois and Minnesota harbored the organism. Cyst stages of amoebae were recovered from the nutrient agar slants prepared in the field, while bacteria grew well. The final sediment of the centrifugation technique yielded individual bacterial colonies and isolated amoebic cysts. The latter were picked out with a sterile loop under the microscope. Cysts thus isolated were placed in a tube containing sterile saline and stored in a refrigerator.

The diphasic liver - peptone - glucose medium was conducive to the axenic growth of the amoebae. A generation time of 25.5 hours was obtained.

All descriptions are based upon observations of living and fixed materials using both conventional light and phase contrast microscopy.

Vegetative stage (Figure 1):

Size—Fifty trophozoites taken at random directly from the latex and from cultures varied from 11 to 34 micra in diameter with an average of 21 micra. It should be realized that size is difficult to measure accurately since the amoebae have variable shapes. A single amoeba may exhibit great variation in size and shape indicated by measurements according to its degree of extension owing to pseudopodial movement.

Pseudopodia—Three types of pseudopods are formed: lobopods, filopods, and

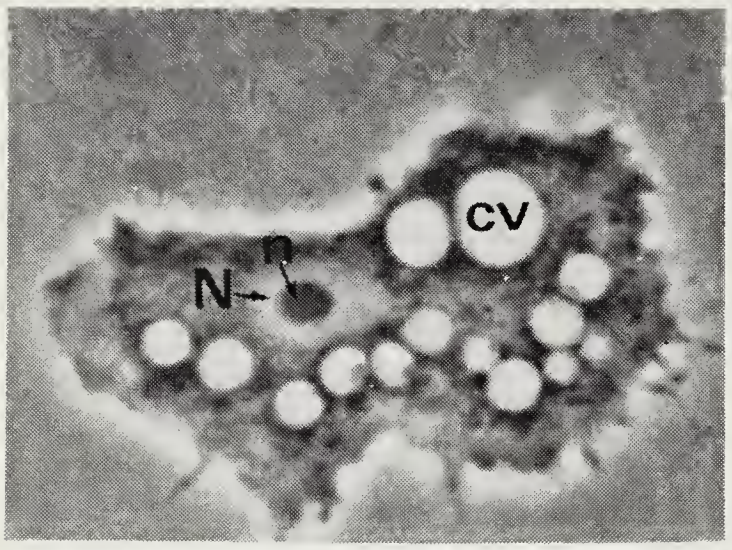

FIGURE 1.—*Hartmannella castellanii* Douglas, living trophozoite from latex of *Euphorbia marginata*. Nucleus (N), nucleolus (n), contractile vacuole (cv). Phase contrast, X 3000.

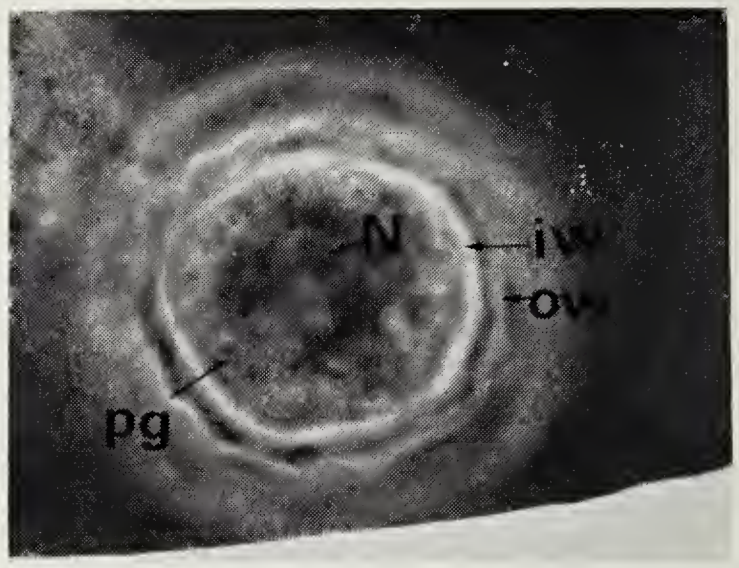

FIGURE 2.—*Hartmannella castellanii*, living mature cyst. Nucleus (N), inner cyst wall (iw), outer cyst wall (ow), peripheral granules (pg). Phase contrast, X 3000.

micropseudopods. All three may be present in the same individual at the same time.

Locomotion — Movement is comparatively sluggish. Differences are observed depending on the type of pseudopods present, the nutritional condition of the culture, and the stage of the life cycle. Prior to encystment, for instance, the amoebae withdraw their pseudopods, become rounded, and cease to move.

Intracellular structure — The cytoplasm, in most cases, is coarsely vacuolated and little cytological detail can be observed. There is a distinct, granular endoplasm and a homogeneous ectoplasm. Numerous filamentous mitochondria are observed upon staining with Janus green. One or more contractile vacuoles are present. No centrosome was observed.

The interphase nucleus is single, spherical, and varies from 3.5 to 4.5 micra in diameter. It possesses a distinct nuclear membrane, a large, very prominent centrally located nucleolus, and peripheral Feulgen positive chromatin granules along the nuclear envelope. The nucleolus is spherical, Feulgen negative, and 1.8 to 2.2 micra in diameter.

Reproduction occurs by mitotic division. No other reproductive phenomena have been observed.

Encystment:

In the laboratory on diphasic LPG medium cysts begin to appear 14 to 16 days after each transfer. The encystment process as observed on isolated amoebae in LPG medium in depression slides lasts anywhere from 25 to 42 hours. At the onset of encystment the amoebae withdraw their pseudopods, become rounded, and cease to move. This precystic form resembles the one described by Ray and Hayes (1954) for *Hartmannella astronyxis*.

The morphology of the mature cyst is similar to that described by Volkonsky (1931) for *Hartmannella castellanii*. The cysts have two outer wrinkled walls and at various places these walls are in contact with each other (Figure 2). There usually is a single row of refractile granules at the periphery just inside the cell membrane. Fifty cysts taken at random from the same sources as the trophozoites varied in size from 11.2 to 13.4 micra with an average of 12.6 micra in diameter.

The description of the latex amoebae agrees very closely with that of an amoeba isolated by Castellani (1930) in a mixed culture of bacteria and *Cryptococcus pararoseus* and now known as *Hartmannella castellanii*.

CONCLUSIONS

On the basis of this study and the excellent discussion by Adam (1964) concerning the taxonomy of hartmannellid amoebae, it is concluded that the amoeba isolated from the latex of *Euphorbia marginata* is *Hartmannella castellanii* Douglas 1930. A synonym is *Acanthamoeba castellanii* (Douglas 1930) Volkonsky 1931. It is noteworthy that this amoeba which in recent years has become a well-known pathogen of

mammalian tissues as well as a contaminant of microbial and tissue cultures is also a plant parasite.

Acknowledgments

This paper represents a portion of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, Department of Zoology, University of Minnesota, Minneapolis, Minnesota. Sincere thanks are expressed to Dr. Franklin G. Wallace, University of Minnesota, for his counsel and assistance and to Dr. Hewson Swift, University of Chicago, for his time and effort assisting with the photomicrography.

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Manuscript received April 11, 1967.

AN ESTIMATE OF ABSOLUTE ZERO — A PHYSICAL CHEMISTRY EXPERIMENT WITH AN INTRODUCTION TO A STATISTICAL TREATMENT OF DATA

R. L. BAIN
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ABSTRACT.—An Experiment is described in which error estimates associated with extrapolated quantities may be made.

The *Chem Study Teachers Guide* [McLellan, 1963, p. 43] suggests that the development of predictive power is one of the rewards of scientic activity. These predictive powers are: repetition, interpolation, and extrapolation. The first of the Chem Study demonstrations is the development of a pressure-temperature relation and an extrapolation of the relation to $P=0$ for an estimate of absolute zero [McClellan, 1963, p. 125]. A further treatment of the data by statistical methods which would give an estimate of the errors associated with the pressure-temperature relation would provide a suitable introduction to a method of estimating errors. Error estimates of quantities obtained from other physical chemistry experiments such as rate constants from slopes or activities from extrapolated EMF measurements may then be made.

The most frequently encountered statistical treatment of data is that of regression analysis [Hald, 1952, p. 522], that is, an analysis which is used to determine the association between two variables in an experiment where one of the variables has little or no error associated with it and whose values are pre-determined when the experiments are planned. Such an estimate of a line on which the data may lie may be obtained by the method of least squares. This is a method of estimating the equation for a line that minimizes the sum of the squares of the deviations from the estimate. Such an estimate is called a regression line. If few observations are taken it may be desirable to associate a certain degree of confidence with an estimate of any error calculated by a least squares method. This degree of confidence may be called a Confidence Limit. Confidence Limits are somewhat arbitrary, but approximately indicate how sure we are that a true value of an observation lies within an indicated range of error. The true value here refers to a statistical value not to an absolute value. Generally, Confidence Limits are tabulated in terms of a probability P and as a function of a distribution called t which is associated with a certain number of degrees of freedom, f, for the observations. The values of t are related to a known error distribution and are used to aid in error estimates.

The equation for the pressure-temperature relation in the demonstration may be approximated by the equation for a straight line since the pressure-temperature changes are obtained at constant volume. Recall that the equation for a straight line through two given points (x_1, y_1) and (x_2, y_2) is:

$$(y-y_1)/(x-x_1) = (y_2-y_1)/(x_2-x_1) \quad (1)$$

rearrangement yields

$$y = y_1 + [(y_2-y_1)/(x_2-x_1)]\ (x-x_1) \quad (2)$$

The quantity $(y_2-y_1)/(x_2-x_1)$ corresponds to the slope called b. The symbol P will represent the pressure (the dependent variable) and the symbol T the temperature (the independent variable).

A suitable experiment may proceed as follows:

A. The pressure is recorded at two accurately known temperatures. The equation for the straight line

through the data points is written and the intercept calculated for the case P=0.

B. The error associated with the pressure-temperature relation is derived by a regression analysis. An error range for the intercept is estimated.

C. The pressure is recorded at a few more accurately known temperatures. A regression analysis is performed, the intercept calculated and an estimate of the range of the intercept for a given set of Confidence Limits is made.

D. Several pressure readings at each accurately known temperature are made and a regression analysis performed as in C.

The temperatures suggested are: near the boiling point of water, room-temperature, an ice-water slush, and a Dry-Ice isopropanol slush. A suitable apparatus is available from Welch Scientific Company, Skokie, Illinois. Typical data and the results of the regression analysis are tabulated in Table 1. An expanded form of Table 1 which includes all pertinent equations and calculations for the data tabulated is available from the author upon request.

It should be pointed out that a statistical treatment of data only gives error information within the range of the observations. Although the apparent error for the intercept decreases with an increase in the total number of observations, reasonably accurate values of quantities desired from extrapolation are often not obtained. The experiment illustrates this point since outside the range of observations the proposed model (the straight line in our case) fails.

This experiment illustrates the advantage of accumulating as many observations as possible and also the hazard associated with extrapolation.

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Manuscript received April 28, 1967.

TABLE 1.—Estimate of absolute zero and associated error estimates

A. Two observations. The equation for a straight line, $y = y_1 + [(y_2 - y_1)/(x_2 - x_1)]\ (x - x_1)$, is used to calculate the intercept when the pressure is zero.

Typical Data:

Temperature, °C	Pressure
$T_1 = 95.0$	$P_1 = 18.5$
$T_2 = -78.5$	$P_2 = 9.5$

$$P = 18.5 + [(9.0)/(173.5)]\ (T - 95.0)$$

$$P = 18.5 + 5.187 \times 10^{-2}\ (T - 95.0)$$

When P is zero, $T_0 = -262°C$

B. The pressure is assumed to have an error and a regression analysis is performed for the two data points. Since two points determine a linear function, there is no statistically calculable error in the equation or intercept.

An estimate of absolute zero based on the data in A together with an estimate of the derived error is $-262°C \pm 0$

C. Four sets of observations, one observation within each set.

95.0	25.0	00.0	−78.5
18.5	15.0	13.5	9.5

$$P = 14.125 + 5.2046 \times 10^{-2}\ (T - 10.375)$$

The intercept at $P = 0$ together an estimate of the error (at 95% confidence limits) is:

$$T_0 = -261°C \pm 14$$

D. Four sets of observations, sixteen total observations.

	Symbol
Temperature	T
Pressure	P

Regression analysis yields the equations for the pressure.

95.0	25.0	00.0	−78.5
18.4	14.9	13.4	9.4
18.5	15.0	13.5	9.4
18.6	15.1	13.5	9.5
		13.5	9.6
		13.6	9.6

$$P = 13.469 + 5.2039 \times 10^{-2}\ (T + 2.0312)$$

The intercept at $P = 0$ together with an estimate of the error (at 95% confidence limits) is:

$$T_0 = -261°C \pm 4$$

INEXPENSIVE CHEMICAL INSTRUMENTATION: Y-TUBE STANDARD CELL

DAVID M. COLEMAN and R. E. VAN ATTA
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Southern Illinois University, Carbondale

ABSTRACT.—Instructions are presented for construction of an inexpensive Weston standard cell, suitable as an internal voltage reference (1.019±0.001 volt) for student potentiometers.

The operation of any student-type potentiometer, such as the Valco model described by Van Atta, Linnenbom, and Coleman (1967), requires standardization of the instrument against a reference voltage source. A reference cell of the Weston, saturated cadmium sulfate type can be easily constructed, by students, from very inexpensive components; it is small enough so that it may be incorporated internally in the Valco potentiometer, making that instrument "self-contained".

MATERIALS

Chemicals required include analytical reagent grade cadmium sulfate, $3CdSO_4 \cdot 8H_2O$, mercury, and mercurous sulfate, Hg_2SO_4. Cadmium shavings may be cut from discs of the metal prepared from pure stick cadmium by the technique described by Vosburgh and Derr (1941). About 4 cm. of B & S 25- gauge platinum or nichrome wire are required for electrical contacts and a 5/16-inch O.D. shell glass Y-tube (about 30 cents each) is used as the cell vessel. The cell may be sealed with commercial epoxy cement.

CONSTRUCTION DETAILS

Seal 2-cm. lengths of the nichrome or platinum wire in the ends of the diagonal arms of the Y-tube, as shown in Figure 1; alternatively, the wire may be cemented in place with epoxy

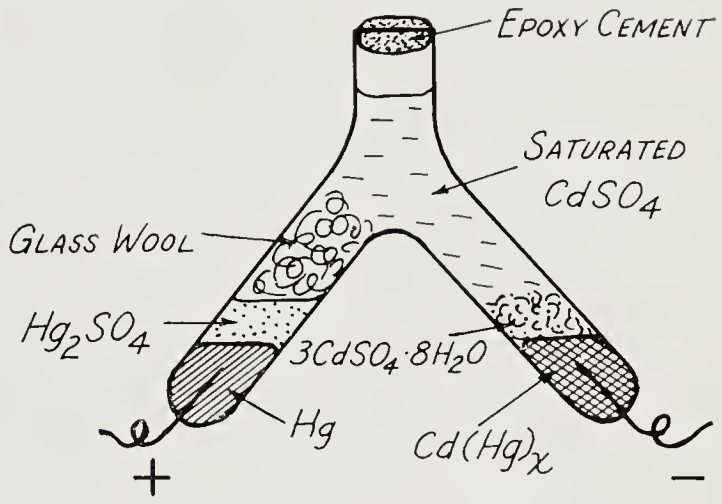

FIGURE 1.—Y tube standard cell.

cement. Score the remaining (upper) end of the Y-tube about 1 cm. above the joint; break off and fire polish the cut end.

Prepare the cadmium amalgam by placing a ½-cm. layer of cadmium metal shavings in the bottom of a 13x100 mm. test tube; add enough pure mercury to just cover the metal, then add about 2 ml. of dilute H_2SO_4 over the mixture. Shake tube and contents in a hot-water bath for one minute, then decant off the excess acid; wash the resulting amalgam thoroughly with distilled water, then transfer a 1-cm. layer of the product into one leg of the Y-tube. Place mercury in the other leg to a similar depth.

Using a small spatula, transfer about 0.5 gram of finely ground Hg_2SO_4 into the mercury leg of the Y-tube; similarly, place about 0.5 gram of finely ground $3CdSO_4 \cdot 8H_2O$ into the amalgam leg. Introduce saturated $CdSO_4$ solution into the Y-tube until the liquid level is just up to the common joint in both legs. With the aid of a stiff wire, force a

small wad of glass wool into the mercury leg, as shown (this will prevent the contents of the two legs from mixing, should the cell be tipped inadvertently). Make certain that there are no large air bubbles in either leg of the Y-tube, then add an additional 0.5 gram of solid cadmium sulfate to the cell.

Seal the top of the Y-tube with epoxy cement and allow the cell to stand for a day or so, after which it is ready for calibration and use.

Performance

More than twenty Y-tube cells have been prepared by high-school and college students in the authors' laboratory. When properly built, these cells have been found to yield potentials of 1.019 $\pm$ 0.002 volt immediately after the addition of the saturated cadmium sulfate solution, stabilizing within a few hours to 1.019 $\pm$ 0.001 volt. Voltages were measured with a Valco student potentiometer, standardized against an Eppley unsaturated cadmium standard cell. Several of the cells have been randomly inverted and shaken from time to time and found to remain stable (within 0.001 volt) over the several months elapsed since their construction. Some have been installed inside the cases of Valco potentiometers and have been found to render highly satisfactory performance as internal reference standards for these instruments.

Acknowledgments

The authors wish to acknowledge the joint support of the National Science Foundation (Grant No. GW-1903) and Southern Illinois University, in connection with the Tenth Secondary Science Training Program for Secondary School Students of High Ability (Summer, 1967). The development and testing of the cells described was conducted, in large part, by high school students participating in that program.

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STUDIES ON THE LATICIFER SYSTEM IN *EUPHORBIA MARGINATA:*

I. LIGHT MICROSCOPIC OBSERVATIONS ON THE DISTRIBUTION OF LATEX VESSELS IN THE EMBRYO

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ABSTRACT. — The distribution of the laticifer system in the embryo of *Euphorbia marginata* is described as a plexus with two components: a stelar component and a cortical-mesophyll component. All the portions of the plexus develop from the laticiferous initials and the branches of these initials located at the periphery of the incipient procambium in the cotyledonary node of the embryo. An earlier researcher, Cameron (1936), concluded that the laticifers of *E. marginata* had an ontogenetic pattern distinct from other members of the Euphorbiaceae studied was in error. His suggestion that two separate latex systems: a stelar and cortical system, exist in the embryo, is corrected and is found to be a single system with the stelar component branching at the cotyledonary node to give rise to the cortical-mesophyll component. The nature of the growth habit and branching of the laticifer plexus of the embryo of *E. marginata* is discussed and illustrated.

Laticifers are cells or series of fused cells that contain latex (Esau, 1965). The latex vessel system found in *Euphorbia marginata* is composed of coenocytic, non-articulated laticifers. This type of multinucleate laticifer originates as a single cell in the embryo and follows the growth of the plant by penetrating into newly formed tissues intrusively between the adjacent cells into various organs of the plant. The non-articulated laticifer cell was observed to become profusely branched at nodal regions during the growth of the plant. Milanez (1952) and Milanez and Neto (1956) reinvestigated the ontogeny of the non-articulated laticifer in *E. phosphorea* and *E. pulcherrima* and concluded from histological studies that the laticifer system was formed from the coalescence of adjacent cells and fusion of their protoplasts to form vesicles. However, Mahlberg (1959) using proliferated embryos of *E. marginata* grown *in vitro* noted that the non-articulated laticifers were not formed by the fusion of cells. The comparison of the three species of *Euphorbia* is valid, but there may be a slight degree of variation in ontogeny of the latex systems.

Cameron (1936) was the first to describe the distribution of the latex system in the embryo of *E. marginata*. He noted that there were two distinct and separate series of latex tubes to be found in these embryos. One, an inner system, was called the stelar latex system and was composed of wide tubes which lie between the vascular bundles in the hypocotyl and radicle. The other system, the outer system, consists of much narrower tubes in the cortex.

These cortical laticifers were found to be unbranched in the hypocotyl and radicle. The stelar and cortical latex systems were found to be connected in the region of the cotyledonary node by a single channel formed by fused cells passing from the stelar latex tubes to the cortical tubes.

Mahlberg (1967) added more information when he noted that in *E. marginata* approximately 12 laticifer initials arose at the periphery of the incipient procambium in the cotyledonary node of the embryo. They appeared at the time of initiation of the cotyledons, and during the subsequent development of the embryo the growing extensions of these cells penetrated along the hypocotyl toward the root meristem and into the enlarging cotyledons. The growing tips, all of which grow between adjacent cells, appear delicate and somewhat pointed, while back from the tip the cells attained a diameter comparable to that of the adjacent cells.

In the present study tissue sections were employed to reexamine and clarify the anatomical distribution of the latex vessels of the embryo of *Euphorbia marginata* and the relationship between the stelar and cortical latex vessels.

Materials and Methods

Seeds of Euphorbia marginata were soaked overnight following which the intact embryos were removed and fixed for 48 hours in Craf IV. The embryos were dehydrated in a tertiary butyl-ethyl alcohol series (Johansen, 1940) and embedded in paraffin. Longitudinal and transverse sections were cut at a thickness of 6 μ. The sections were stained in Heidenhain's hematoxylin and counterstained with 1% safranin in 50% ethyl alcohol.

Observations

The distribution of the laticifers in the embryo (Fig. 1) can be described as a complex system of branched non-articulated latex vessels forming a single system of latex vessels all of which arose from the 12 laticiferous initials found at the periphery of the procambium in the cotyledonary node of the embryo. During subsequent development the growing extensions of these initials illustrated a bidirectional elongation. At one end the initial penetrated along the long axis of the hypocotyl toward the root meristem and at the other end of the initial the penetration is into the enlarged cotyledons. These tubes which comprise the major stelar components of the laticifer send out numerous horizontal branches.

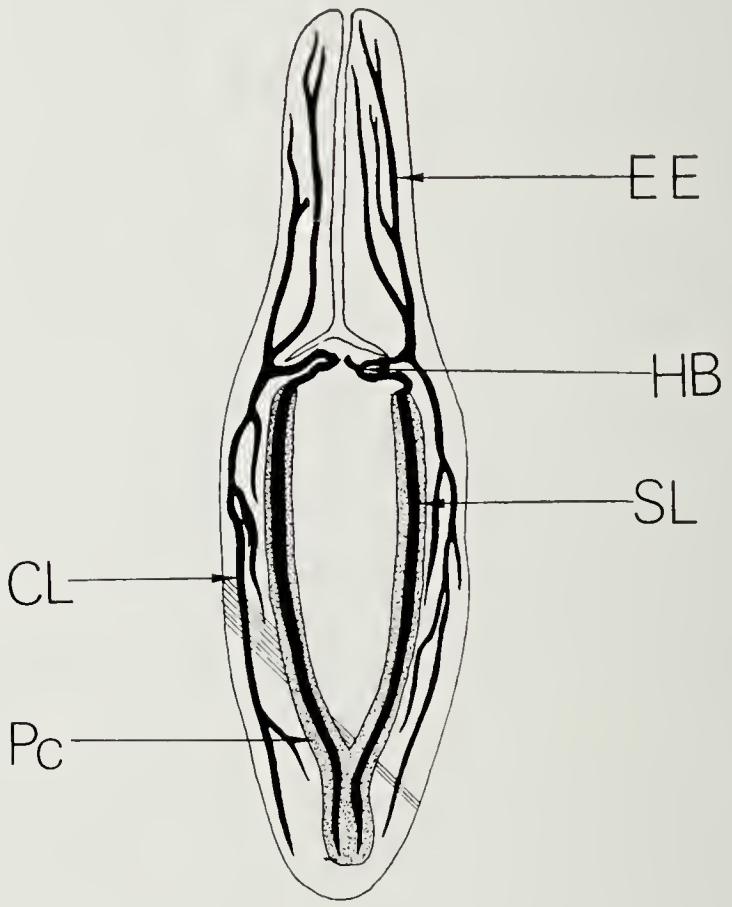

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Figure 1. Camera lucida drawing of the embryo of *Euphorbia marginata* to show distribution of the latex system resulting from the development of two of the 12 lacticiferous initials.

C.L. cortical latex component of the hypocotyl

S.L. stelar latex component of the hypocotyl

Pc procambium in hypocotyl

H.B. horizontal latex tubes and branches in the cotyledonary node.

E.E. laticifer extensions in the mesophyll of the epicotyl

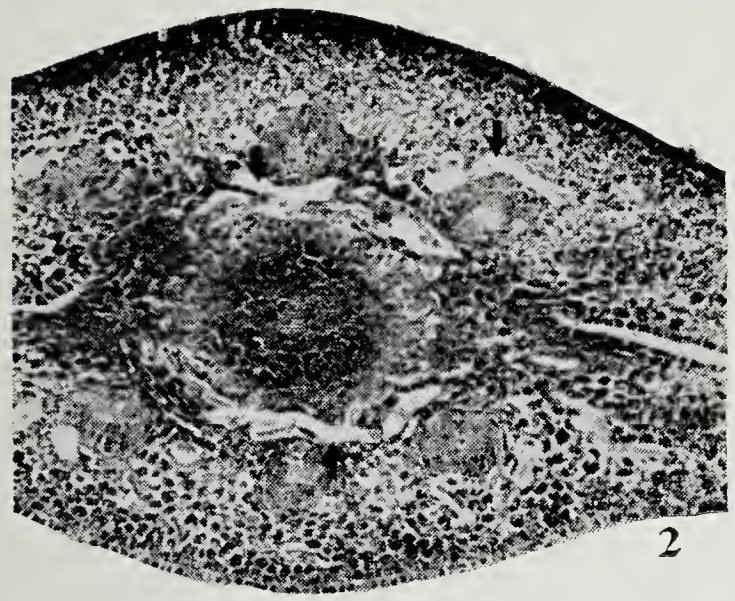

FIGURE 2. Transverse section through region of cotyledonary node showing horizontal branching of latex vessels.

These horizontal branches appear in the cotyledonary nodal region (Fig. 2) and some of these branches are transstelar. The laticifer branches at the cotyledonary node bifurcate repeatedly sending out a network of branches into the mesophyll of the cotyledons and other branches into the cortical region of the hypocotyl (Fig. 3). This network of tubes found in the mesophyll of the cotyledons and the cortex of the hypocotyl are narrower than the stelar component of the laticifer system.

In the embryo the laticifers were easily detected; starch grains were not seen in the laticifers under the light microscope as in the other cell types of the embryo, protein and lipid inclusions are abundant. In the seedling the opposite is observed: a large number of starch grains are seen in the latex vessels whereas protein and lipid inclusions disappear.

In a transverse section of the hypocotyl six vascular bundles are observed. The stelar and cortical elements of the latex system are distinct in this region (Fig. 4). The cortical elements appear as a number of isolated cells occurring below the epidermis up to a depth of about five cells and are absent from the remaining cell layers of the inner cortex. The stelar latex system consists of about 12 fairly wide cells two or three of which are present on either side of each vascular bundle. As the hypocotyl is examined there is relatively little change in the positions of the 12 stelar vessels. As the hypocotyl tapers off and has a narrower cortex, the stelar and cortical components are brought closer together, but they remain distinct; no anastomoses between any of the latex vessels was observed. The latex vessels terminate as pointed tips just above the root apex.

As the cotyledons expand and mature, laticiferous vessels follow the vascular bundles and branch profusely (Fig. 5) sending out numerous branches into the mesophyll of the cotyledons. No branches appeared in the pith region of the hypocotyl.

DISCUSSION

In *Euphorbia marginata* the distribution of the latex vessels is similar to the pattern in other members of the Euphorbiaceae (Chauveaud, 1891). However, there is no evidence to substantiate the claim of Cameron (1936) that two separate systems: a stelar system and a cortical laticifer system exists. Rather this researcher has found that a single initial gives rise to branches at the cotyledonary node which profusely branch and grow into the cortex of the hypocotyl and mesophyll of the cotyledons.

This research effort provided still more evidence to support the interpretation of non-articulated laticifers as distinct and individual cells (Chauveaud, 1891). The interpretation of the origin of the non-articulated laticiferous system within the young embryo ascribed to occurrence of cellular fusion (Cameron, 1936: Milanez and Neto, 1956) was refuted by Mahlberg (1959) and is supported by this work.

Utilizing ultrastructural techniques it may be possible to obtain considerable data on cellular growth and development on specialized cells such as the non-articulated laticifers. Such studies on the laticifers of *E. marginata* are in progress.

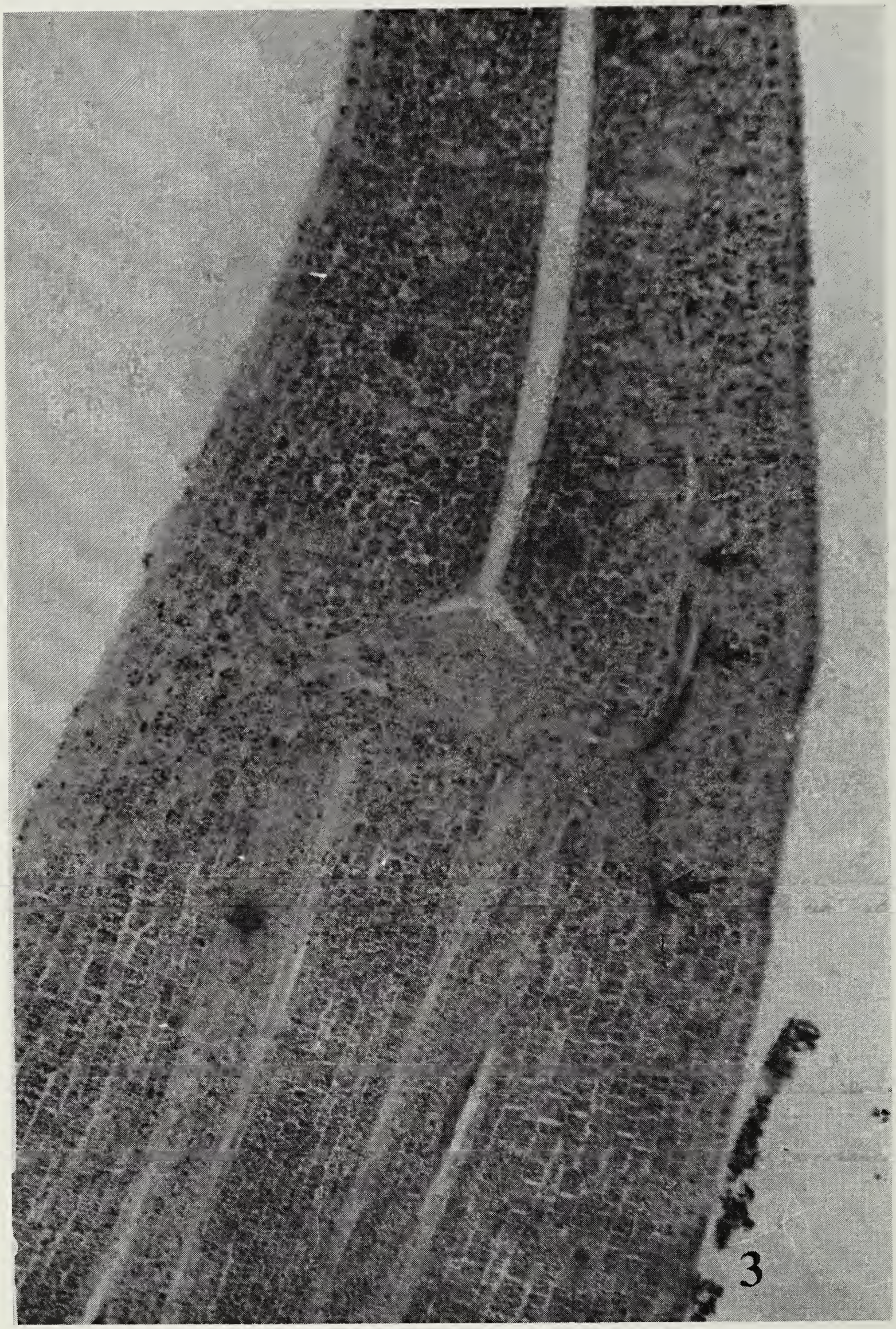

FIGURE 3. Longitudinal section of embryo showing branches from cotyledonary node oriented to form the cortical-mesophyll component of the latex system.

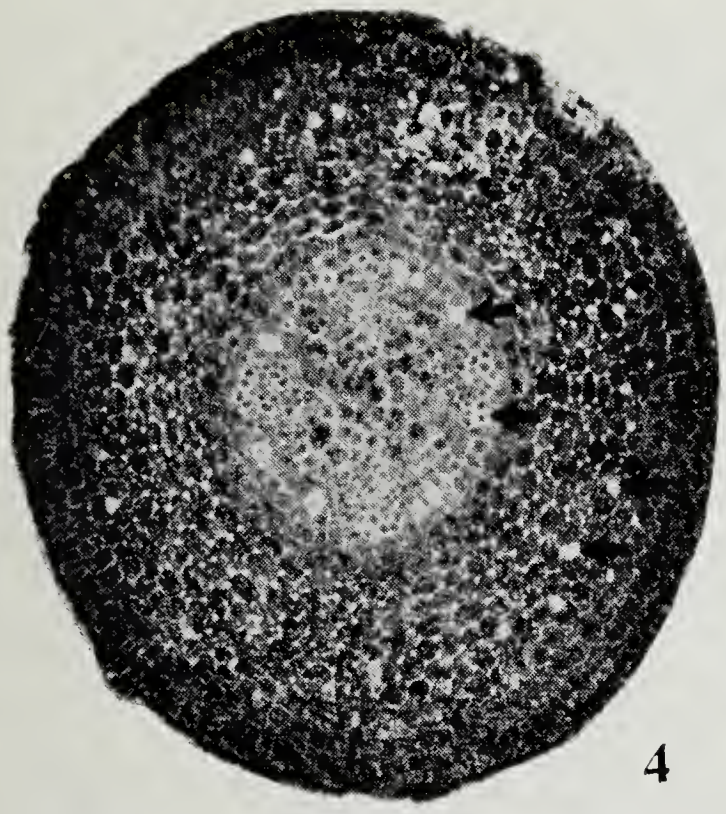

FIGURE 4. Transverse section through the hypocotyl showing the outer cortical component and the inner stelar components of the latex system.

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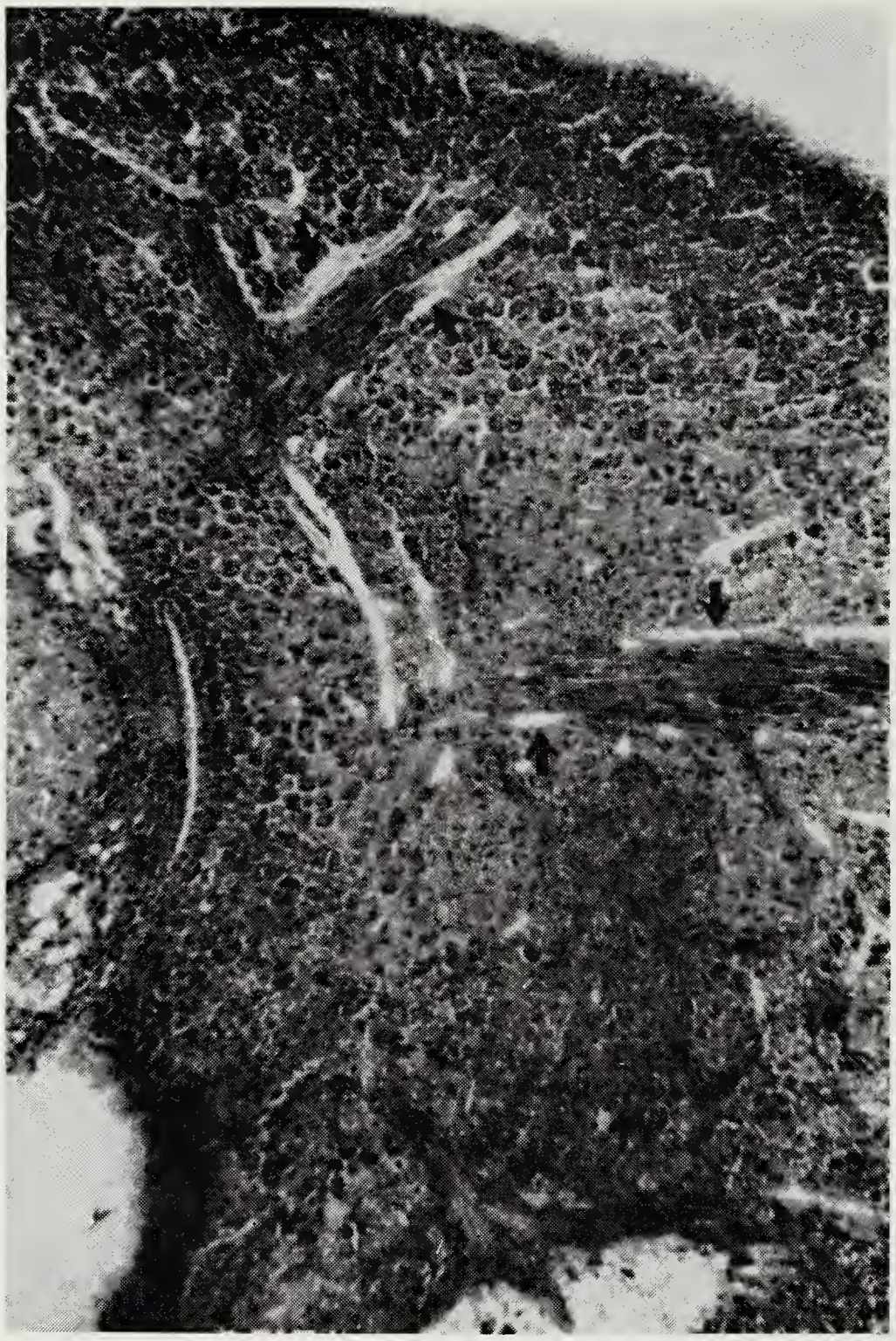

FIGURE 5. Longitudinal section through the cotyledon showing the branched latex vessels in close proximity to the branches of the vascular tissue.

AVAILABILITY AND USE OF MEDICAL FACILITIES: A GEOGRAPHIC CASE STUDY

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ABSTRACT. — Using Golconda, Illinois as the research area, this study determines, from a sample survey of households, (i) the percentage of the population that visited selected health facilities during 1966, and (ii) where the population traveled to these facilities.

The number of medical facilities represents an important component of every region's health program. Brown (1966) noted that the physician-population ratios in underdeveloped countries may range from 1 to 20,000 to 1 to 100,000. In developed countries the ratio is around 1 to 1,000 (Sanders, 1965). Another component is the degree of use of these facilities. It has been found in several studies that availability of health facilities may not correspond to their use. For example:

> "The National Health Service in Great Britain is one of the most comprehensive health services for the population ever devised. Yet, in a study of a representative sample of families in London, 37 percent of the families (as reported by the mothers) had a member suffering pain or discomfort and not being treated. A visit to a physician is free of charge" (Anderson, 1965, p. 363).

Thus, together the availability and use of health facilities in an area form two keys to public health programs. This paper seeks to reveal through a case study, how a geographic analysis may add to an understanding of these two keys.

Specifically, using Golconda, Illinois, in Pope County, as the research area (Figure 1), this exploratory study is concerned with determining:

(i) The availability of medical facilities for the Golconda population. The medical facilities in question are those of the physician, dentist, and hospital.

(ii) The use of these facilities. By use, I mean whether or not a member of a household has visited a physician, dentist, or hospital.

(iii) The patterns of movement to the medical facilities.

The local data on availability and use are compared with national data, taken from the Health--Household Interview Survey conducted by the U.S. National Health Survey during the early 1960's.

METHODS

Selection of the site. — Golconda is located in the Quadri-County area of southern Illinois where the University of Illinois Center For Zoonoses Research is conducting its investigations. (This is a multi-disciplinary investigation of diseases common to man and other animals.) This study is part of

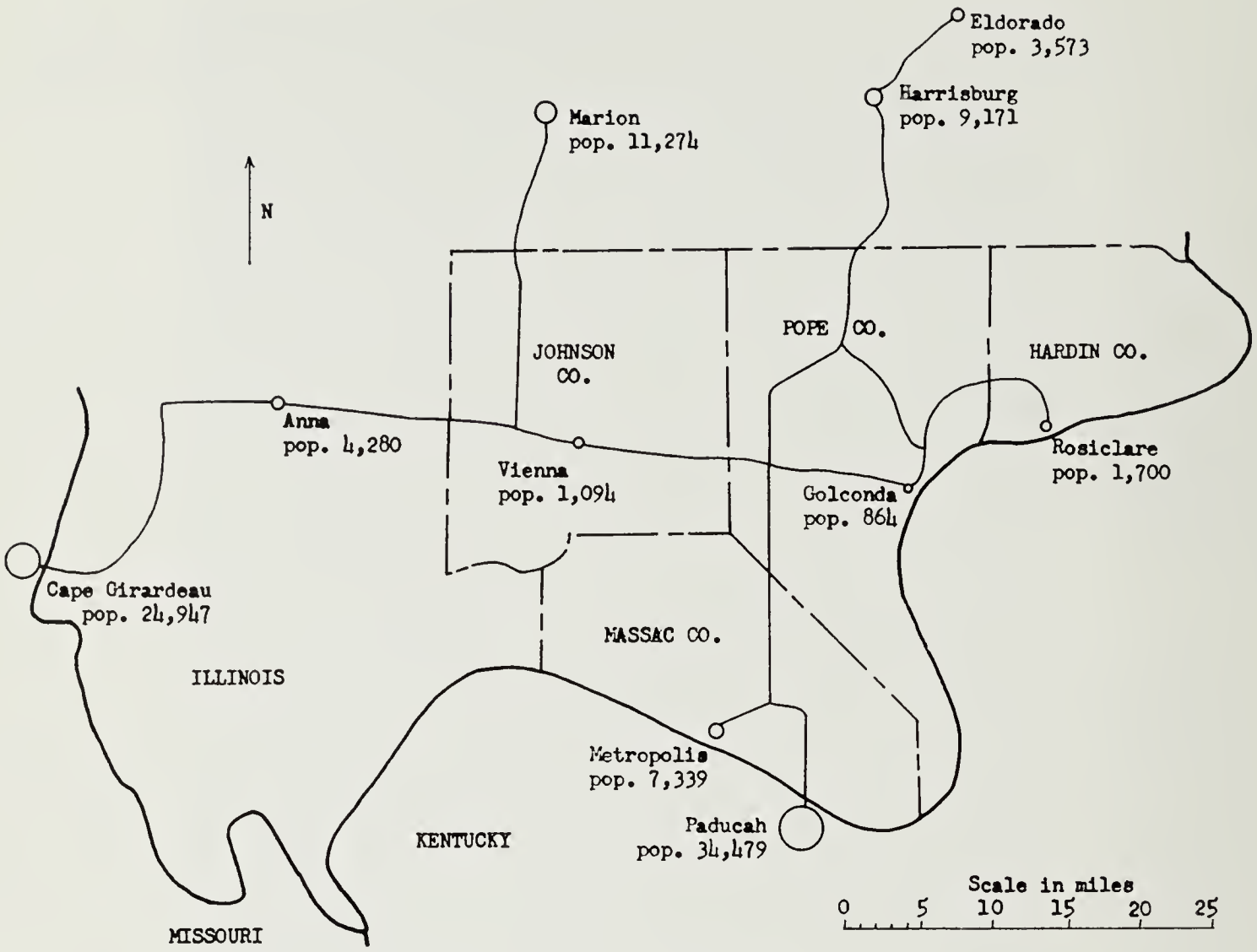

FIGURE 1. — Southern Illinois Study Area.

that work. There are, however, more specific reasons for the selection of Golconda. The size of the town (864 in 1960) was sufficiently large to have (for the area) a representative population in terms of sex and age structures, yet the town was not so large that it could not be studied in the short time available. Also, with the small number of medical facilities in the town (a physician and a dentist), it was assumed that many of the inhabitants would go elsewhere for medical attention. Finally, there was not much difference in distance to four other towns—Metropolis, Rosiclare, Paducah, and Harrisburg — each of which had a greater number of medical facilities than Golconda.

Sample design. — The sample population was drawn from within the legal town boundaries of Golconda. A sampling fraction of one-fifth, or every fifth household, was used. The town was arbitrarily divided into three sampling units of approximately equal area and number of households. Three random numbers, drawn from a random numbers table, were used as the starting points for each unit. For instance, in unit one, "4" was the random number. I started at the fourth household and then interviewed every fifth household thereafter in that sampling unit, always beginning at the northwest corner of each block and working clockwise. The purpose of this procedure was to avoid possible "linear trends" (Stahl, 1966).

The total sample population was 136 inhabitants. This constituted 63 households, of which 58 responded. There were two refusals and three vacancies. Two recalls were made to the vacancies at different times on different days. Then, neighbors were asked to confirm the vacancies. A total of only four people constituted the number of refusals. Adding those four inhabitants to the total expected population from the vacant households (2.3 inhabitants per household times 3 households equals 6.9, or rounded off to 7) it was found that the non-response constitutes 7.9 percent (i.e., 11/147) of the total possible response.

A Chi-square test (2x2 contingency table) was used to test the internal consistency of the sample population data. Two random samples were drawn and found not to be statistically significant (hypothesis accepted $H_o = H_1$) at the 0.05 level of significance.

The value for the number of inhabitants per household, as indicated above, 2.3, was below the average for the county (2.92 for 1960). This is probably due to the fact that there are a large number of elderly persons living alone in the town. This large elderly population in Golconda is evidenced in the age structure of the sample population, which is heavily weighted above 65 years of age (Figure 2).

Data. — A questionnaire was used for each member of the household. Information was sought on: age, sex, visits to the physician, dentist, hospital, and where the person went for these services. The information concerned only the past year, January to December, 1966. It was hoped that memories would still be relatively strong for this time period.

Regarding the comparisons with national findings, statistical analyses, e.g., chi-square, have not been used, since the data from my survey and the national survey were collected at different times, not co-ordinated areally, and were vastly different in size of sample. Therefore, the comparisons should be treated only as tentative indicators.

Results

Availability of medical facilities. —The question posed on availability was: is the ratio of medical facilities to population in the Quadri-County area equal to the state and national ratios? As can be seen in Table I, the answer is no. For all three services, physician, dentist, and hospital, the Pope County and Quadri-County ratios were far below the state and national ratios. The lack of a physician in Johnson County is particularly notable. Focusing on Pope County and Golcon-

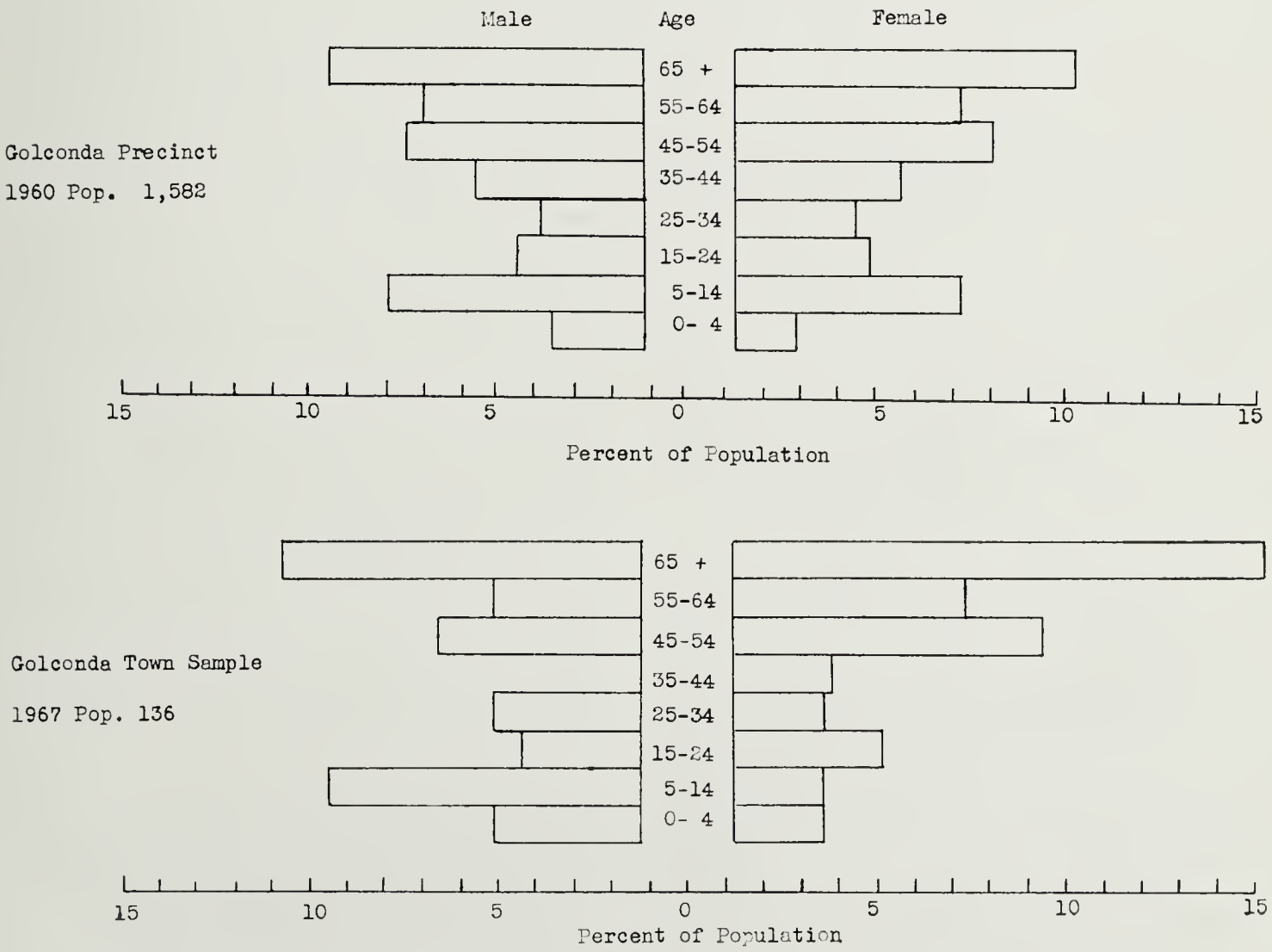

FIGURE 2. — Age-Sex Structures.

TABLE 1.—Availability of Medical Facilities in the Quadri-County Area, the State of Illinois and the United States.

Quadri-County Area[1]

County	Population	Physician (P)	(P) per 100,000 Popu.	Dentist (D)	(D) per 100,000 Popu.	Hospital	Hospital Beds (Hb)	(Hb) per 1,000 Popu.
Hardin	5,879	2	66	2	34	1	27	4.6
Johnson	6,928	.....	.....	2	29	.....	.....	.....
Massac	14,341	6	42	4	28	1	60	4.2
Pope	4,061	1	25	1	25	.....	.....	.....
Total	31,209	9	29	8	26	2	87	2.8

Illinois[2] and United States[3]

Area	Physician per 100,000 Population	Dentist per 100,000 Population	Hospital Beds per 1,000 Population
Illinois	137	60	4.2
United States	143	52	3.8

[1] Field study, January, 1967.
[2] U.S. Department of Health, Education, and Welfare (1965, p. 5-15).
[3] Pennell and Baker (1965, pp. 26-27).

da, several people expressed their concern to me over the need for another physician. And they were also becoming concerned about the future need for a dentist, as the present one is old and declining in health.

Use of medical facilities. — The data on visits to physicians appear to indicate a similarity between the Golconda and national averages (Table II). In both cases about two-thirds of the population visited the physician at least once during a one-year period. Also in each case, a lower percentage of men than women visited the physician.

For dental visits, the Golconda percentage was generally below the national percentage (Table III). Comparisons cannot be made with national differences between sexes, since recent data on a national scale are not available. The Golconda data indicate that females visited the dentist more often than males. This indication is in accord with previous national experience (Anderson, 1956, p. 198).

There was a striking difference between the percentage of hospital visits on the local and national levels (Table IV). While appoximately 20 percent of the Golconda sample pop-

TABLE 2.—Persons with a Physician Visit within a Year by Sex and Age: Golconda, Illinois. (January 1966-December 1966)[1] and United States (July 1963-June 1964)[2]

Age	Both Sexes			Male			Female		
	Gol.[3] No.	Gol. %	U.S. %	Gol. No.	Gol. %	U.S. %	Gol. No.	Gol. %	U.S. %
All ages	91	66.9	66.1	40	61.5	62.7	51	71.8	69.3
Under 5 years	10	83.3	80.4	5	71.4	81.7	5	100.0	79.1
5-14 years	11	61.1	61.2	7	53.8	61.5	4	80.0	60.8
15-24 years	7	53.8	66.1	3	50.0	60.7	4	57.1	71.0
25-34 years	8	66.7	66.2	4	57.1	57.3	4	80.0	74.3
35-44 years	1	16.7	63.4	0	.....	58.2	1	20.0	68.3
45-54 years	16	72.7	63.4	6	66.7	58.7	10	76.9	67.9
55-64 years	12	70.5	63.6	5	71.4	60.2	7	70.0	66.8
65-74 years	10	76.9	67.9	4	100.0	63.3	6	66.7	71.1
75+ years	16	69.5	70.5	6	54.5	67.7	10	83.3	72.6

[1] Field study, January, 1967.
[2] National Center for Health Statistics. "Physician Visits." (1965, p. 29).
[3] Gol. no. refers to the actual number of visits to the physician, from Golconda sample.

TABLE 3.—Persons with a Dentist Visit within a Year by Sex and Age: Golconda, Illinois (January 1966-December 1966)[1] and United States, (July 1963-June 1964).[2]

Age	Both Sexes			Male			Female		
	Gol. No.	Gol. %	U.S. %	Gol. No.	Gol. %	U.S. %	Gol. No.	Gol. %	U.S. %
All ages	45	33.3	42.0	20	30.7	N.A.	25	35.0	N.A.
Under 15 years	11	36.7	39.5	8	40.0	.....	3	30.0	.....
15-24 years	10	76.9	55.2	4	66.7	.....	6	85.7	.....
25-44 years	7	38.9	58.5	4	50.0	.....	3	30.0	.....
45-64 years	13	33.3	38.4	4	25.0	.....	9	39.1	.....
65+ years	4	11.1	20.8	0	.....	.....	4	19.0	.....

N.A.—Data was not available.
[1] Field study, January, 1967.
[2] National Center for Health Statistics. "Current Estimates." (1965, p. 19).

TABLE 4.—Persons with a Hospital Visit within a Year by Sex and Age: Golconda, Illinois, (January, 1966-December 1966)[1] and United States (June 1960-July 1962)[2].

Age	Both Sexes			Male			Female		
	Gol. No.	Gol. %	U.S. %	Gol. No.	Gol. %	U.S. %	Gol. Nol	Gol. %	U.S. %
All ages	27	19.9	9.3	15	23.0	7.0	12	17.0	11.4
Under 15 years	7	23.3	5.0	5	23.8	5.6	2	20.0	4.3
15-24 years	3	23.1	12.5	2	33.3	5.1	1	14.3	19.1
25-44 years	5	27.8	12.2	2	25.0	6.3	3	30.0	17.7
45-64 years	6	15.4	9.5	3	18.8	9.5	3	13.0	9.5
65+ years	6	16.7	11.2	3	20.0	11.8	3	14.3	10.7

[1] Field study, January, 1967.
[2] National Center for Health Statistics, "Persons Hospitalized." (1965, p. 15)

ulation visited a hospital, only about 10 percent of the national sample population did so. Further, in Golconda more males than females visited the hospital, exactly the opposite of the national sample findings.

Location of, and movement to, medical facilities.—Only about one-third (of those visiting) of the sample population visited the Golconda physician (Table V). The percentage of males and females staying in Golconda for medical services was almost exactly the same—one-third. No clear patterns emerged as to age-location distinctions. A combined total of over 50 percent of the patients went to Paducah, Rosiclare, and Metropolis. The high percentage of visits to Paducah is explained, in large part, by the medical specialists found there. The close proximity of Rosiclare to Golconda appears to account, in part, for its percentage of visitors.

The percentage of visitors to the local physician, as contrasted with visitors to the local dentist, is striking: almost ¾ of those visiting the dentist stayed in Golconda, while only about 1/3 visited the local physician. Why is there this difference? Excluding the possibility of a large sampling error, I suggest that psychological factors are responsible. In short, what several people told me was simply, "I don't like him," in referring to the local physician.

Since Golconda has no hospital, the inhabitants must go elsewhere for this medical facility. Rosiclare stands out as the most important center, getting 1/3 of the Golconda hospital visitors. Paducah, which has the largest number of beds and technical equipment in the immediate area, received one-quarter of the visitors. For special services, more distant centers were used: Evansville,

TABLE 5.—Medical Service Visitors from Golconda to Selected Centers.[1]

Center	Physician		Dentist		Hospital	
	Number	%	Number	%	Number	%
Quadri-County Area						
Golconda	38	33.9	32	71.1	0	.....
Metropolis	15	13.3	5	11.1	5	17.2
Rosiclare	20	17.8	1	2.2	10	34.5
Vienna	1	0.8	.....	.....	.....	.....
Illinois: Other						
Anna	0	.....	0	.....	0	.....
Danville	0	.....	1	2.2	2	6.8
Eldorado	2	1.7	0	.....	0	.....
Harrisburg	5	4.4	4	8.8	2	6.8
Marion	1	0.8	0	.....	1	3.4
Indiana						
Evansville	0	.....	0	.....	1	3.4
Kentucky						
Paducah	28	25.0	0	.....	7	24.1
Missouri						
Cape Girardeau	1	0.8	0	.....	0	.....
St. Louis	0	.....	0	.....	1	3.4
Tennessee						
Murfreesboro	1	0.8	1	2.2	0	.....
Total	112	99.3	45	99.8	29	99.6

[1] Field study, January, 1967.

St. Louis, and, for veterans, Marion and Danville, Illinois.

CONCLUSIONS AND SUGGESTIONS

Certain general points emerge from the results of this exploratory study. First, the medical facilities of Golconda in particular, and of the entire Quadri-County area, appear to be inadequate for the given population, as noted in the population-medical facility ratios. Additionally, psychological factors play a large part in reducing the effectiveness of the Golconda physician. Furthermore, one of the Quadri-County dentists works only part time, and will presumably retire within a few years.

Second, the Quadri-County area has long been characterized by both a small and declining population and a low income level. These conditions are not conducive to enticing more medical facilities and personnel into the area.

Third, despite these conditions, in general it appears that the population did visit the physician as often as the national sample population. The percentage of the Golconda hospital visitors was even above the national percentages.

Fourth, (Table V) the Quadri-County medical facilities received well over half of the Golconda visitors. Thus, the facilities of the area are, indeed, important to the inhabitants of Golconda.

Concerning future work in the Quadri-County area I would suggest the following:

Regarding availability of medical facilities, four approaches should be considered. First, Golund's work in Sweden (1961) and Kuhinka's work in Pennsylvania (1965) have revealed that time-distance factors are important in determining areas of inadequate service in relation to hospitals. I suspect that for the most part few places in the Quadri-County area would be beyond Kuhinka's limit of 30 minutes by car from a local hospital; but the point is worth validating. Second, a precise definition of available medical facilities in centers should be established. Cross-checks should be made with physicians to determine their specialties and with hospitals to determine the extent of their facilities. Third, ignoring measures of quality, the numerical sufficiency of facilities could be more vigorously determined through two measures: the total number of visitors to each facility, and the percentage of the total population using the facilities, as determined by a household survey. Fourth, other medical facilities should be considered, particularly the Quadri-County Health Department.

Regarding the use of medical facilities, I would propose an extension of the household sampling approach, since that approach can provide fairly precise answers to the questions of who uses what facilities, how often, where, and why.

In particular, explanations of patterns of movement to medical facilities need to be considered not only in terms of distance, number and type of facilities, and size of centers, but also in terms of the household decision-maker's value structure. In this vein, Koos (1954) found that a calculus of economic, psychological, and sociological factors determine what facility a person will visit.

Finally, I suggest that the household survey would be a valuable means by which to involve specific populations in the continual processes of planning and redevelopment (Shimkin, 1965). Thus, hopefully, a long-term mutual concern could develop on the part of both the medical personnel and the population on the effectiveness of availability and use of medical facilities.

Acknowledgments

I wish to thank Mr. John Cippola and his Golconda staff of the Quadri-County Health Department, Dr. Paul Schnurrenberger of the Illinois Department of Public Health, and Dr. Robert Kokernot of the Center for Zoonoses Research, for their generous assistance.

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Manuscript received June 20, 1967.

INFLUENCE OF SOLUBLE CARBOHYDRATE CONTENT AND PROTEIN LEVEL ON *IN VITRO* CELLULOSE DIGESTION IN ORCHARDGRASS

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ABSTRACT.—Twenty-three forage samples were fermented *in vitro* to determine the effect of soluble carbohydrate content on cellulose digestion. To observe the effect of soluble sugar content on lag period (differential rate in the beginning of cellulose digestion), two groups of samples, one group containing 6% and the other 10.5% soluble carbohydrate, were fermented *in vitro* for six hours. No significant difference could be shown in the extent of cellulose digested between the two groups of forages. Similar analysis also showed there was no significant difference in cellulose digestion (at 12 or 24 hours) due to soluble carbohydrate content.

Nitrogen fertilization significantly increased cellulose digestion at both 12- and 24-hour fermentation times. Initial cellulose content in the nitrated forages was significantly lower than in the non-nitrated forages.

The animal agriculture in much of southern Illinois and adjoining south central states depends in large measure on grazing ruminants (cattle and sheep). The efficiency of cattle and sheep production is dependent upon the production of high quality forage. Many forage crops have been studied singly and in mixtures in an effort to produce maximum forage yields. In many cases the legumes in legume-grass mixtures are short-lived and thus the establishment of pure stands of grasses is common. Orchardgrass (Dactylis glomerata) is one of the most popular high producing perennial grasses used. Little information is available on the nutritive value of orchardgrass especially under grazing conditions.

Progress in the development of *in vitro* techniques has stimulated interest in the application of this method in the evaluation of forage quality. Recently, many workers have found forage cellulose digestion *in vitro* to be highly correlated with the digestibility of various forage constituents *in vivo* (Baumgardt *et al.*, 1959; Hershberger *et al.*, 1959; Donefer *et al.*, 1960; Le Fevre and Kamstra, 1960; Reid *et al.*, 1960; Baumgardt *et al.*, 1962; Simkins and Baumgardt, 1963). There is little doubt that *in vitro* rumen fermentation techniques offer a method of studying the digestibility and thus nutritive value of forages.

Crampton (1957) suggested level of intake (voluntary consumption) of a forage may be of primary importance in describing its feeding value. This observation was extended and in 1959 Macdonald College workers (Crampton *et al.*, 1959; Donefer *et al.*, 1959) introduced the use of *in vitro* cellulose digestion as a possible means of estimating the total nutritive value of forages.

Donefer *et al.* (1959) observed lag periods in the start of cellulose digestion of 2 to 6 hours between forages. This differential rate of fermentation was reflected in 12-hour cellulose digestion which in turn was highly correlated (r = 0.88) with *ad libitum* consumption *in vivo*. Cellulose digestibility at 24 hours was highly correlated (r = 0.87) with *in vivo* energy digestibility.

Since rumen microorganisms benefit (in terms of cellulose digestion) from a readily available source of carbohydrate, it could be postulated that a close relationship exists between level of soluble carbohydrates in forages and the initiation of rapid *in vitro* cellulose digestion. If a close relationship does exist it may be that level of soluble carbohydrate could be used to predict voluntary intake of the forage.

A series of trials was conducted by Reid *et al.* (1964) to determine the influence of growth phase (first vs. regrowth), stage of maturity, nitrogen fertilization (60 lb. N vs. 240 lb. N) and cutting management (continuous growth vs. interval clipping) on digestibility of Kentucky bluegrass. In Reid's study it was noted that higher levels of nitrogen fertility increased cellulose digestibility *in vitro* and *in vivo* in the first growth phase of grass. It is possible that this effect due to nitrogen is related in part to the amount of nitrogen available to the microorganisms.

In the study reported in this paper *in vitro* fermentation is used to assess the effects of the soluble carbohydrate and nitrogen content of orchardgrass on cellulose digestion.

Materials and Experimental Procedures

Orchardgrass (Dactylis glomerata) samples used in this investigation were obtained from the Lawrence area of land at the Dixon Springs Agricultural Center, Simpson, Illinois.

The Lawrence tract, consisting of 40 acres, is of the Grantsburg-Grenada silt loam soil type. After being in corn and winter wheat, the area was seeded in the spring of 1959. The field was cut for hay once in 1959, twice in 1960 and once in 1961. The area became a permanent pasture in the fall of 1961. After being divided into two equal parts, ammonium nitrate was applied at the rate of 209 pounds per acre in the spring of 1962 and 106 pounds per acre in the fall to the north half. In October 1962, soil tests indicated the pH to be 6.57 for the south half and 5.83 for the north half, the latter value apparently a result of fertilization, since the pH prior to dividing the field was 6.30. Lime was applied at the rate of 0.3 ton per acre to the south half and 2.3 ton per acre to the north half. In 1963 fertilizer applications were: 228 pounds ammonium nitrate per acre to the north half on April 12 and 111.6 pounds per acre on August 20; 204.4 pounds of super phosphate per acre on the north half and 207.7 pounds per acre on the south half on August 29.

Orchardgrass samples were collected weekly from two locations (east and west ends) in each half of the field. Forage samples were obtained directly from the grazed area by clipping a four foot square plot. Plots were located at random within each end of the field. Only selected samples taken from the west ends, representing the period from October 29, 1962, to December 30, 1963, were used in the present study. A visual estimate indicates there was very little variation in the forage stand and that 90-95% of the growth was orchardgrass. There was no attempt made to evaluate the extent or type of disease.

The grazing season includes the period from the third week of April to the third week of October. During the 1963 grazing season, "put and take" animals were used to insure an equal degree of grazing on both halves of the field.

Forage samples were dried in a forced draft oven at 60° C, and dry matter content calculated. Macro - Kjeldahl

techniques were used to determine protein content. In preparation for sugar extraction and *in vitro* digestion work, forage samples were ground using an intermediate Wiley mill, equipped with a 60-mesh screen.

Two basic approaches were used in evaluating the effect of soluble carbohydrates and protein content of orchardgrass on cellulose digestion. The first involved an extraction and colorimetric determination of the soluble sugars in forage samples. The second phase of this study was the use of an *in vitro* technique to determine cellulose digestibility.

A number of techniques for the extraction of sugars present in forages are reported in the literature. Techniques involving the use of ethanol as the extracting solvent have been commonly used. With this is mind a modification of a method used by Gilmore (1964) was chosen for this study. Following a 6-hour extraction with 70% ethanol and removal of impurities and color pigments, hydrolysis of the di-and oligosaccharides (non-reducing) was carried out enzymatically with a 1% diastase solution. After standing overnight, the colorimetric method for determining sugars, described by Dubois *et al.* (1956), was used.

That phase of the study to be reported in this paper involves three factors: (1) the effect of soluble carbohydrate level on the initial lag period in forage cellulose digestion; (2) the effect of nitrogen fertility on the rate and extent of cellulose digestion; (3) the relationship between level of soluble carbohydrate and extent of cellulose digestion.

With these interests in mind, a careful selection of materials for study was necessary since the sample size, representing each date, was small. Twelve samples, six having around 6% soluble carbohydrate, and six containing around 10% were selected to study lag phase. These selections contained enough forage material to carry out duplicate *in vitro* digestions at 6, 12 and 24 hours. Donefer *et al.* (1959) observed lag periods of 2-6 hours in the start of cellulose digestion. Thus differences in the length of lag phase between forages would be reflected in the 6-hour digestion time. To study the effect of nitrogen fertility eight pairs (2 samples collected on the same date, one from the north half of the field, receiving nitrogen, and one from the south half) were selected. *In vitro* cellulose digestion was determined at 12 and 24 hours. The combined selections (samples from parts 1 and 2) are used to study the third relationship. In all, 23 samples were selected for *in vitro* digestion studies.

Cellulose digestion *in vitro* was determined by the fermentation technique described by Bentley *et al.* (1955), with modifications introduced by Johnson *et al.* (1958), Quicke *et al.* (1959) and Donefer *et al.* (1960). For the sake of completeness and clarity the modified technique used in the present study is described.

Fermentation was carried out in 32 x 200 mm. (125 ml.) pyrex test tubes placed in a water bath thermostatically controlled at 39° C. Tubes were equipped with rubber stoppers fitted with glass inlet and outlet tubes to permit the passage of CO_2 throughout the fermentation period.

Approximately 1 gm. of the air-dry test forage was placed in each tube. *In vitro* cellulose digestion was determined on each forage in duplicate for all fermentation times (6-, 12-, 24-hour), within the same fermentation run. The tubes were located throughout the fermentation system at random.

The buffer-mineral solution used in the present study was that used by Baumgardt *et al.* (1962) and was similar to the synthetic saliva suggested by McDougall (1948). The composition is shown in Table 1. Before each fermentation run this solution was bubbled vigorously with CO_2 until the pH was approximately 6.9.

A mature ewe, fitted with a permanent ruminal canula and maintained on good quality, coarsely ground orchardgrass hay, served as the source of rumen microorganisms for all fermentations. Inoculum preparation was according to a modification of the method described by Johnson *et al.* (1958). Liquor, first expressed from the rumen

TABLE 1. Buffer-Mineral Solution for *In Vitro* Fermentation

Component	*Gm. Per Liter*
$NaHCO_3$	9.8
$Na_2HPO_4 \cdot 7H_2O$	7.0
KCl	0.57
NaCl	0.47
$CaCl_2 \cdot 2H_2O$	0.053
$MgSO_4 \cdot 7H_2O$	0.12

contents through several layers of cheesecloth, was discarded. The remaining pressed pulp was extracted with phosphate buffer (2.000 gm. $Na_2HPO_4 \cdot 7H_2O$, 0.436 gm. KH_2PO_4 per liter, pH 7.0, prewarmed to 40° C) in the ratio of 454 grams pressed pulp to 375 ml. of buffer. This mixture was re-expressed through cheesecloth and the fluid thus obtained put immediately into prewarmed thermos jugs. This phosphate buffer extract was used as the inoculum.

Inoculum pH values ranged from 7.0 to 7.25 over the fermentation periods. The tubes were inoculated as rapidly as possible (about 10 minutes) with 10 ml. of the phosphate buffer extract. The CO_2 supply was adjusted so that moderate bubbling occurred in the tubes. The pH was determined following each fermentation. Even though pH adjustments were not made pH did not drop below 6.4 in 24 hours. It is generally considered cellulose digestion takes place efficiently down to a pH of 6.5 (Barnett and Reid, 1961). The pH level reported here presumably would not have any marked effect on bacterial activity.

At the end of the fermentation period the microbial activity was stopped by addition of 1 ml. of 4N H_2SO_4 to each tube.

Cellulose was determined by the gravimetric method of Crampton and Maynard (1938) as modified by Hershberger *et al.* (1955). Where possible all cellulose determinations were carried out in duplicate, i.e., initial cellulose and residual cellulose after fermentation *in vitro*.

To account for variations between *in vitro* fermentation periods a reference forage (fescue) was fermented during each period.

The data were statistically analyzed by the method of least squares. Both simple and multiple regressions were also run on selected data.

Results and Discussion

A total of 116 orchardgrass samples, collected during a period from October 29, 1962, to December 30, 1963, were analyzed for soluble carbohydrate content. Marked fluctuations in level of soluble carbohydrate content in the forage were observed during the period studied. This observation is in agreement with that of other investigators (Sullivan and Sprague, 1943; Crampton and Jackson, 1944; Barnett and Miller, 1950; Sprague and Sullivan, 1950; Ely *et al.*, 1953; Waite and Boyd, 1953a, b; Waite, 1957; Van Riper and Smith, 1959; and Stoddardt, 1964). Although several trends in forage soluble carbohydrate content due to the effect of ammonium nitrate applications were observed, no significant differences could be shown.

The results of 23 *in vitro* fermentations are presented in Table 2. Percent protein, initial cellulose and soluble carbohydrate for each sample are included. Statistical analysis of the data for the 6-, 12-, and 24-hour fermentations revealed no significant effect on 6-hour cellulose digestion due to the level of soluble carbohydrate in the forage. Similar analysis also showed that there was no effect at 12 or 24 hours although the F value at 24 hours was approaching significance. The mean soluble carbohydrate and *in vitro* cellulose digestibilities and standard deviations are presented in Table 3. Although there was no significant effect of level of soluble carbohydrate on the extent to which cellulose was digested *in vitro* it should be noted that at all time periods greater amounts of cellulose were digested in forage samples containing the higher levels of soluble carbohydrates. It can be concluded from these results that a source of readily available energy was apparently not a limiting factor so far as microbial activity was concerned in the forages studied.

TABLE 2.—*In Vitro* Cellulose Digestion—23 Orchardgrass Samples.

Date of Cutting	Location	Protein (%)	Initial Cellulose (%)	Soluble Carbohydrate (%)	Cellulose Digestion		
					6-Hour (%)	12-Hour (%)	24-Hour (%)
11- 5-62	N ½	21.35	25.39	6.36	24.90	38.95	56.95
11- 5-62	S ½	14.00	31.42	4.91	.....	19.45	34.00
11-12-62	N ½	20.65	21.84	8.12	.....	22.03	49.62
11-12-62	S ½	16.45	27.64	6.31	16.15	23.87	39.24
12-10-62	N ½	16.80	25.54	6.78	.....	32.47	55.02
12-10-62	S ½	13.83	27.13	6.39	13.88	19.60	40.29
1- 7-63	N ½	15.93	27.94	6.30	17.02	27.89	38.12
1-21-63	N ½	17.85	30.82	6.18	13.27	30.90	45.54
1-21-63	S ½	8.40	36.72	6.59	.....	11.58	23.67
1-28-63	N ½	16.45	32.26	6.87	.....	25.84	42.33
1-28-63	S ½	13.13	41.30	5.67	2.31	8.60	16.11
4-15-63	N ½	23.28	23.86	12.79	.....	51.94	70.81
4-22-63	N ½	25.20	25.39	8.40	.....	54.80	69.69
4-22-63	S ½	15.58	26.77	10.76	12.21	25.98	51.35
4-29-63	S ½	11.90	26.83	10.03	14.44	19.31	49.53
5-13-63	N ½	20.48	30.63	8.35	.....	47.19	63.35
5-13-63	S ½	11.90	31.36	9.67	.....	20.79	49.30
5-20-63	N ½	19.78	27.85	10.92	18.06	35.90	57.05
5-20-63	S ½	12.78	30.94	10.85	15.63	26.95	42.42
6- 3-63	N ½	22.93	27.91	10.50	22.81	41.01	53.04
7-15-63	S ½	.....	35.50	10.16	12.55	21.29	40.20
8-19-63	N ½	.....	35.16	8.95	.....	20.60	35.03
8-19-63	S ½	.....	39.69	8.55	.....	33.82	45.59

TABLE 3.—Influence of Level of Soluble Carbohydrates on *In Vitro* Cellulose Digestion at 6, 12, and 24 Hours.

Level of Soluble Carbohydrates %		Cellulose Digestion					
		6-Hour %		12-Hour %		24-Hour %	
Mean	S[a]	Mean	S	Mean	S	Mean	S
6.2	0.8	14.6	7.3	25.0	10.3	39.3	13.3
10.5	0.4	15.9	4.0	28.4	8.4	49.0	6.5

[a] Standard deviation.

Other workers have noted various responses in digestibility due to the application of nitrogen fertilizer on several forages. Hall *et al.* (1958) compared utilization of nitrated (120 lb. N per A) and non-nitrated forages at two stages of maturity using *in vitro* cellulose digestion and observed that nitrogen fertilizer at the pasture stage did not affect the utilization of millet or piper sudan, whereas it had a marked negative effect on the utilization of sweet sudan. No differences in utilization were observed at the hay stage. Markley *et al.* (1959) found increased apparent digestibility coefficients *in vivo* of protein and energy of bromegrass and orchardgrass when at least 200 lb. of nitrogen per acre were applied. In *in vitro* work, Reid *et al.* (1964) showed a significant increase in cellulose and protein digestion due to nitrogen fertility. The effect of nitrogen on cellulose digestion *in vitro* can be found in Table 4. Nitrogen application did have the effect of significantly increasing cellulose digestion of orchardgrass at both 12- and 24-hour fermentation times. Reid *et al.* (1964) made the suggestion that

TABLE 4.—Influence of Nitrogen Fertilization on the *In Vitro* Digestion of Cellulose at 12 and 24 Hours.

Nitrogen Fertility	Level of Crude Protein %		Level of Soluble Carbohydrates %		Cellulose Digestion			
					12-Hour %		24-Hour %	
	Mean	S[a]	Mean	S	Mean	S	Mean	S
+	19.8	2.85	7.75	1.55	36.0	10.9	54.9	9.3
0	13.2	2.45	7.65	2.38	24.9	6.5	37.0	12.1

[a] Standard deviation.

this effect may be related in part to a limitation in nitrogen availability for cellulose digestion in the *in vitro* growth medium. Other workers (Chalupa *et al.*, 1963) have noted significant stimulatory effects on cellulose digestibility when low levels of urea were added to a rumen fluid phosphate buffer medium. Upon further examination of data obtained in this investigation, it was noted the cellulose content of the nitrogen series was significantly lower than in the non-nitrogen series. This would suggest the possibility the level of cellulose present or other materials associated with various levels of cellulose in forage may be an important factor governing its rate of digestion. The authors question that the levels of cellulose per tube used in this study would cause the differences observed.

It was also of interest to obtain the relationship between level of soluble carbohydrate and extent of cellulose digestion. Correlation coefficients at three *in vitro* digestion times are presented in Table 5. It will be noted rather low correlations were obtained, especially at 6 and 12 hours, between percent soluble carbohydrate and percent cellulose digestion. The correlation improved as fermentation time increased ($r = 0.60$ at 24 hours). According to Donefer *et al.* (1959) cellulose digestion at 12 hours is closely associated with *ad libitum* consumption and 24-hour cellulose digestion is closely associated with forage digestibility. Bowden (1964), reported simple correlations of 0.60 to 0.69 between soluble carbohydrate content of orchardgrass and 48 hour *in vitro* dry matter digestion. This report appears to agree closely with the present results at a 24-hour digestion time.

Simple correlations between percent nitrogen and percent cellulose digestion were high at all three digestion times ($r = 0.72$ to 0.87). Also from the correlations made it appears that there was a definite negative association at all digestion times studied between initial cellulose and percent cellulose digestion ($r = -.55$ to $-.78$). These results would indicate the nitrogen content

TABLE 5.—Correlation Coefficients Between 3 Forage Components and Cellulose Digestion *In Vitro*.

Factors Correlated	6-Hour Digestion r(11 samples)	12-Hour Digestion r(20 samples)	24-Hour Digestion r(20 samples)
% protein *AND* % cellulose digestion	0.72*	0.87**	0.77**
Initial cellulose *AND* % cellulose digestion	—.78*	—.55*	—.75**
% soluble carbohydrate *AND* % cellulose digestion	0.24	0.44	0.60**
Multiple correlation coefficient	0.91**	0.90**	0.90**

** P = 0.01
*P = 0.05

and the amount of initial cellulose have a greater effect on digestion of cellulose *in vitro* than the amount of soluble carbohydrate present in the forage, especially early in the fermentation period. It further appears that use of all three dependent variables predicts cellulose digestion more accurately than any one variable alone since a multiple correlation coefficient provided values of 0.90 for all three digestion times. This means that approximately 81% of the variation in cellulose digested can be accounted for by including the variation in soluble carbohydrate, nitrogen and initial cellulose. Bowden (1964) found he could account for approximately 60% of the variation in dry matter digested *in vitro* when water soluble carbohydrate, protein and fructose were included in a multiple regression analysis.

Close examination of Table 2 suggests that there may be other factors involved which influence cellulose digestion *in vitro*. For example, samples collected on November 12, 1962, (N ½) and May 13, 1963, (N ½) have approximately the same protein and soluble carbohydrate content. Even though initial cellulose was higher in the May sample, it was digested to a greater extent in 24 hours. Generally, cellulose digestion values were observed to be higher in forage samples collected during the spring and summer than in those collected from November to January. This would imply that, in addition to the influence of the forage components studied on cellulose digestion, there may be confounding of this response due to season. This, quite possibly, is associated with the degree of lignification in the forage. Kamstra *et al.* (1958) reported a negative relationship between lignin content and cellulose digestion *in vitro*. In the present study, determination of the lignin content of forages, may have been extremely useful in explaining variations in cellulose digestion *in vitro*; however, limited sample precluded this.

Acknowledgment

The authors wish to express appreciation for the assistance of Dr. H. W. Norton in the statistical analysis and interpretation of the data obtained.

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Manuscript received May 16, 1967.

CONTRIBUTIONS TO AN ILLINOIS FLORA NO. 2
COMPOSITAE I. (TRIBE VERNONIEAE)

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ABSTRACT.—Seven taxa of *Vernonia* and one of *Elephantopus* are recorded from Illinois. Keys, descriptions, and distributional maps are provided for each taxon.

The tribe Vernonieae is composed of perennials with alternate leaves. The flowers are tubular, perfect, and purple (rarely pink or white). The style branches are filiform, acute or acuminate, and hispidulous. The anthers are distinctly sagittate. The pappus is composed of capillary bristles or scale-like bristles or both.

The tribe is represented in Illinois by two genera, *Vernonia* and *Elephantopus*. *Vernonia* comprises a genus of about 500 species widely distributed in all tropical areas and extending into temperate regions in North and South America, being most numerous in tropical America. The Illinois flora contains five species of *Vernonia*, one of which may now be extinct in the state. *Elephantopus* is composed of about 18 species distributed in the tropical and warm regions in both hemispheres, with a single species occurring in Illinois.

SYSTEMATIC TREATMENT

Key to the Illinois Genera of Vernonieae

1. Pappus double, the inner of rough capillary bristles, the outer of scales or short bristles; heads not glomerate .....1. *Vernonia*
1. Pappus a single series of bristles; heads glomerate, subtended by sessile bracts .....2. *Elephantopus*

1. VERNONIA Schreb. Gen. Pl. 2:541. 1791.

Suprago Gaertn. Fruct. 2:402. 1791.
Baccharoides Moench. Meth. 578. 1794.
Ascaricida Cass. Dict. Sci. Nat. 3: suppl. 38. 1816.
Lepidaploa Cass. Bull. Soc. Philom. 1817:66. 1817.
Achyrocoma Cass. Dict. Sci. Nat. 26:61. 1823.
Senecioides Post & Kuntze, Lex. Gen. Phan. 2:515. 1823.

Erect perennial herbs; stems leafy, branching, glabrous or pubescent; leaves alternate, simple; inflorescence corymbiform; heads discoid, 13-to many-flowered, ebracteate; involucre subcylindrical to hemispheric, urceolate; phyllaries imbricate, inner progressively longer, persistent, spreading at maturity; receptacle flat to subconvex; corolla regular, 5-cleft, purple (rarely pink or white); anthers sagittate, syngenesious; style-branches gradually attenuate, minutely hispidulous outside; pappus double, outer of short capillary or scale-like bristles, inner of longer capillary bristles; achene ribbed, pubescent or glabrous, usually resinous.

Key to the Illinois Species of *Vernonia*

1. Phyllary tips long-filiform; heads 12-20 mm wide.....1. *V. crinita*
1. Phyllary tips obtuse to abruptly acuminate; heads 4-12 mm wide.
   2. Leaves tomentose to tomentellous beneath.
      3. Phyllary tips obtuse to mucronate, erect or slightly spreading ....2. *V. missurica*
      3. Phyllary tips abruptly acuminate, squarrose ..3. *V. baldwinii*
   2. Leaves glabrous or scabrous-hirtellous beneath (sometimes tomentellous on veins).
      4. Leaves glabrous, conspicuously punctate beneath; outer pappus of short capillary bristles .....4. *V. fasciculata*
      4. Leaves scabrous-hirtellous, scarcely punctate beneath; outer pappus of scale-like bristles .....5. *V. altissima*

1. VERNONIA CRINITA Raf. New Fl. 4:77. 1838.

Vernonia arkansana DC. Prodr. 7:264. 1838.

Cacalia arkansana (DC.) Kuntze, Rev. Gen. 969. 1891.

Erect perennial herbs; stems 1-3 m tall, simple or sparingly branched, leafy, glabrous or minutely puberulent; leaves linear to linear-lanceolate, 8-18 cm long, 5-20 mm wide, acuminate, tapering to base, entire or finely serrulate-denticulate, thin, glabrous above, glabrous or sparsely pubescent and impressed-unctate beneath; inflorescence irregular, with 5-40 heads; peduncles stout, thickened above, 1-5 cm long; heads 55-to-90 flowered; flowers purple; involucre 10-18 mm high; phyllary tips long-filiform, erect or spreading, glabrous or pubescent; pappus purple to brown, inner bristles 6-7 mm long, outer similar, but shorter; achenes 5-6 mm long, strongly ribbed, glabrous or subglabrous, usually resinous in furrows; $n=17$.

Vernonia crinita is found in open woods and prairies from Missouri to Kansas, Oklahoma, and Arkansas, being adventive in Ohio and Illinois. It is known to have occurred in Illinois at a single locality in Champaign County which has since been destroyed by a construction project: At edge of road west of Champaign about a quarter of a mile south of the Champaign Airport, August 3, 1955, *Slimmer 111* (ILL); September 18, 1955, *Jones 20598* (ILL); September 12, 1957, *Winterringer 13829*, 13830 (ISM); September 30, 1958, *Winterringer 14851* (ISM). (Fig. 1).

2. VERNONIA MISSURICA Raf. Herb Raf. 28. 1841.

Key to the Illinois Forms of *Vernonia missurica*

1. Flowers purple ....2a. *f. missurica*
1. Flowers rose or white.
   2. Flowers rose .....2b. *f. carnea*
   2. Flowers white ....2c. *f. swinkii*

2a. VERNONIA MISSURICA Raf. f. MISSURICA

Vernonia fasciculata Michx. var. βT. & G. Fl. N. Am. 2:58. 1841.

Vernonia altissima Nutt. var. *grandiflora* A. Gray, Syn. Fl. N. Am. 1:90. 1884.

Vernonia drummondii Shuttlew. in Werner, Jour. Cinc. Soc. Nat. Hist. 16:171. 1894.

Vernonia interior Small var. *drummondii* (Shuttlew.) Mack. & Bush, Man. Fl. Jackson Co. 190. 1902.

Vernonia illinoensis Gleason, Bull. N. Y. Bot. Gard. 4:211. 1906.

Vernonia reedii Daniels, Univ. Mo. Stud. Sci. 1:364. 1907.

Vernonia michiganensis Daniels, Univ. Mo. Stud. Sci. 1:402. 1907.

Vernonia missurica Raf. var. *austroriparia* Gleason, Bull. Torrey Club 46:245. 1919.

Erect perennial herbs; stems 1.0-1.5 m tall, branched above, leafy, tomentellous; leaves lanceolate to ovate-lanceolate, 6-15 cm long, 1.5-6.0 cm wide, long-acuminate, acute to rounded at base, subentire to sharply serrate, firm, scabrellate above, thinly to densely tomentose beneath; inflorescence open, flattened or concave, 1-5 cm wide; heads 34-to 55-flowered; flowers purple; involucre broadly campanulate, short-cylindrical to hemispherical, 6-8 mm high; phyllary tips rounded to mucronate, appressed, imbricate, purple or greenish along the midvein, glabrous or puburulent and resinous on the back, entire or arachnoid-ciliolate; pappus tawny to purple, inner bristles 6-8 mm long, outer bristles broader and short-

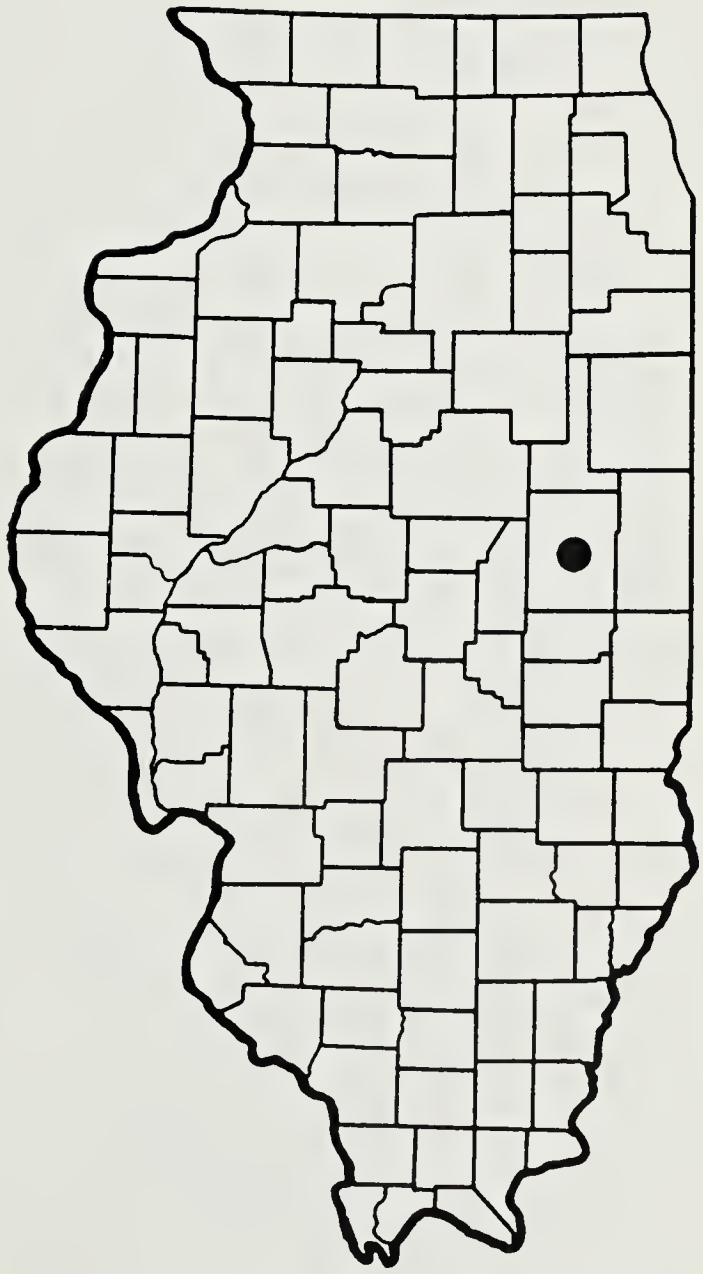

FIGURE 1. Distribution of *Vernonia crinita* in Illinois.

er; achenes 4.0-4.5 mm long, subglabrous to puberulent on ribs, resinous in furrows; $n = 17$.

Vernonia missurica f. *missurica* is found in open woods and prairies from Ontario to Iowa, south to New Mexico, and east to Alabama. It is common throughout Illinois (Fig. 2). It exhibits considerable variation, primarily in leaf pubescence and the inflorescence. The species tends to approach *V. altissima* by the presence of short, conical, multicellular hairs on the lower leaf surface and in the more loose inflorescence with smaller heads. A number of taxa have been named from local races within the species as is noted by the extensive synonomy. *Vernonia illinoensis* has been considered by some as a hybrid between *V. missurica* and *V. altissima* and even to some extent to be derived from *V. baldwinii* and *V. fasciculata*. The author regards these individuals so assigned to be variations within the species and, therefore, places them in synonomy under *V. missurica*.

2b. VERNONIA MISSURICA Raf. f. CARNEA Standley, Rhodora 32:33. 1930.

This form differs from *V. missurica* f. *missurica* in having its flowers rose-colored rather than purple.

It is known only from one collection in Illinois: PEORIA CO.: *Chase 3266* (ILL.) (Fig. 3).

2c. VERNONIA MISSURICA Raf. f. SWINKII Steyermark, Rhodora 59:24. 1957.

This form differs from *V. missurica* f. *missurica* in having its flowers white rather than purple.

It is known only from the type locality and from one collection in Illinois: COOK CO.: Prairie south of 95th Street and west of US 45 in Argonne Forest Preserve south of Willow Springs, *Swink 2775* (F.) (Fig. 4).

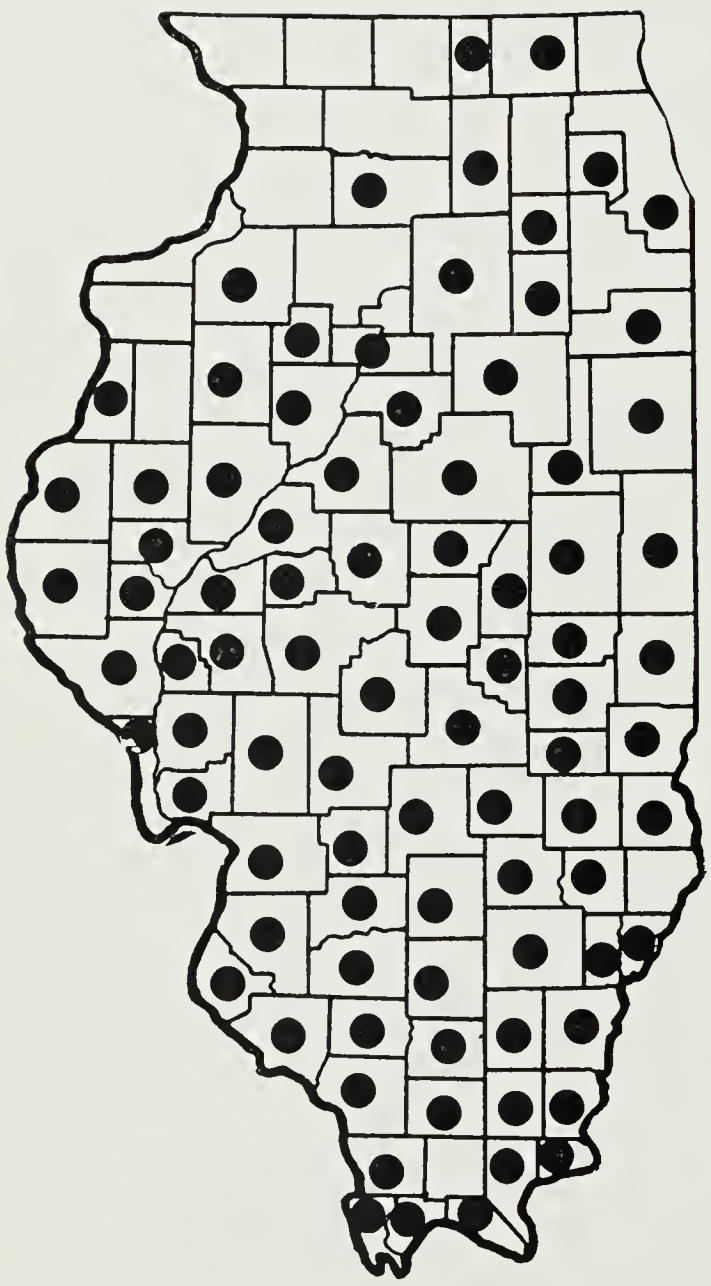

FIGURE 2. Distribution of *Vernonia missurica* f. *missurica* in Illinois.

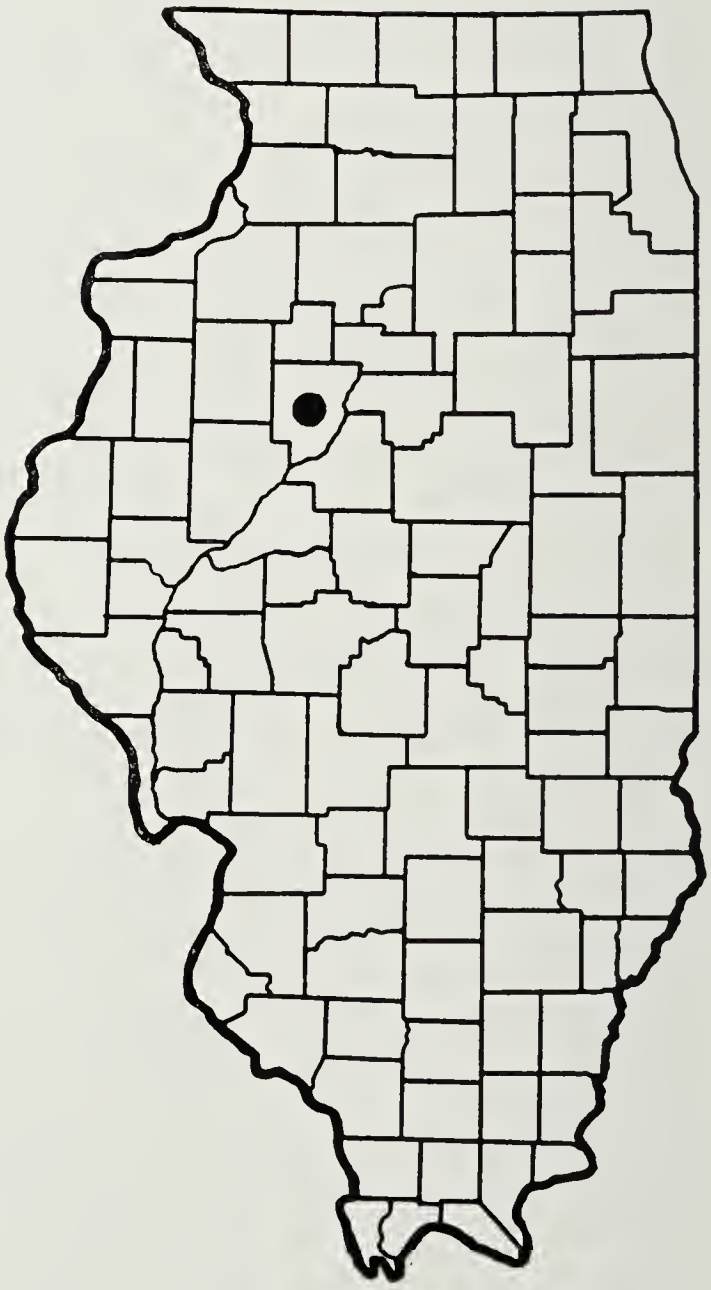

FIGURE 3. Distribution of *Vernonia missurica* f. *carnea* in Illinois.

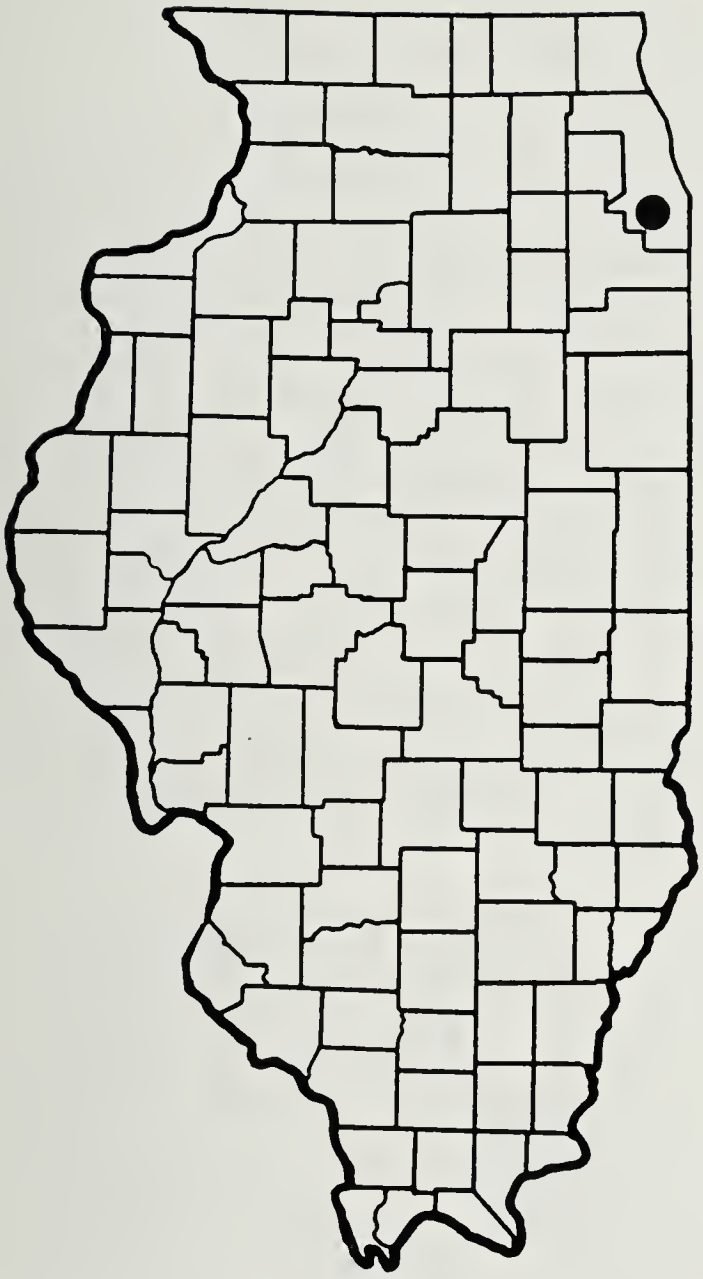

FIGURE 4. Distribution of *Vernonia missurica* f. *swinkii* in Illinois.

3. VERNONIA BALDWINII Torr. Ann Lyc. N. Y. 2:211. 1827.
Vernonia sphaeroidea Nutt. Trans. Am. Phil Soc. II 7:283. 1840.
Cacalia gigantea Kuntze var. *baldwinii* (Torr.) Kuntze, Rev. Gen. 324. 1891.
Cacalia baldwinii (Torr.) Kuntze, Rev. Gen. 969. 1891.
Vernonia interior Small, Bull. Torrey Club 27:279. 1900.
Vernonia interior Small var. *baldwinii* (Torr.) Mack. & Bush. Man. Fl. Jackson Co. 190. 1902.
Vernonia duggariana Daniels, Univ. Mo. Stud. Sci. 1:360. 1907.
Vernonia flavipapposa Daniels, Univ. Mo. Stud. Sci. 1:360. 1907.
Vernonia parthenioides Daniels, Univ. Mo. Stud. Sci. 1:360. 1907.
Vernonia prealta Daniels, Univ. Mo. Stud. Sci. 1:362. 1907.
Vernonia pseudobaldwinii Daniels, Univ. Mo. Stud. Sci. 1:363. 1907.
Vernonia baldwinii Torr. var. *interior* (Small) Schubert, Rhodora 38:370. 1936.

Erect perennial herbs; stems 0.5-1.5 m tall, branched above, leafy, tomentellous to tomentose; leaves lanceolate to ovate-lanceolate, 8-15 cm long, 2.5-5.0 cm wide, acuminate, narrowed to acute base, sharply serrate, firm, puberulent above, tomentellous and resinous-dotted beneath; inflorescence open, irregular, 1-2 dm wide; heads 18- to 35-flowered; flowers purple; involucre campanulate to thick-cylindrical, 5-7 mm high; phyllary tips acuminate, squarrose or reflexed, appressed, imbricate, green with purple margin, puberulent and resinous on back, arachnoid-ciliolate; pappus tawny to light purple, inner bristles 6 mm long, outer bristles broader and shorter; achenes 3 mm long, puberulent, resinous in furrows; $n = 17$.

Vernonia baldwinii is found in prairies and open ground from Illinois to Oklahoma to Arkansas. It is local in the southern two-thirds of Illinois (Fig. 5). This species also exhibits consider-

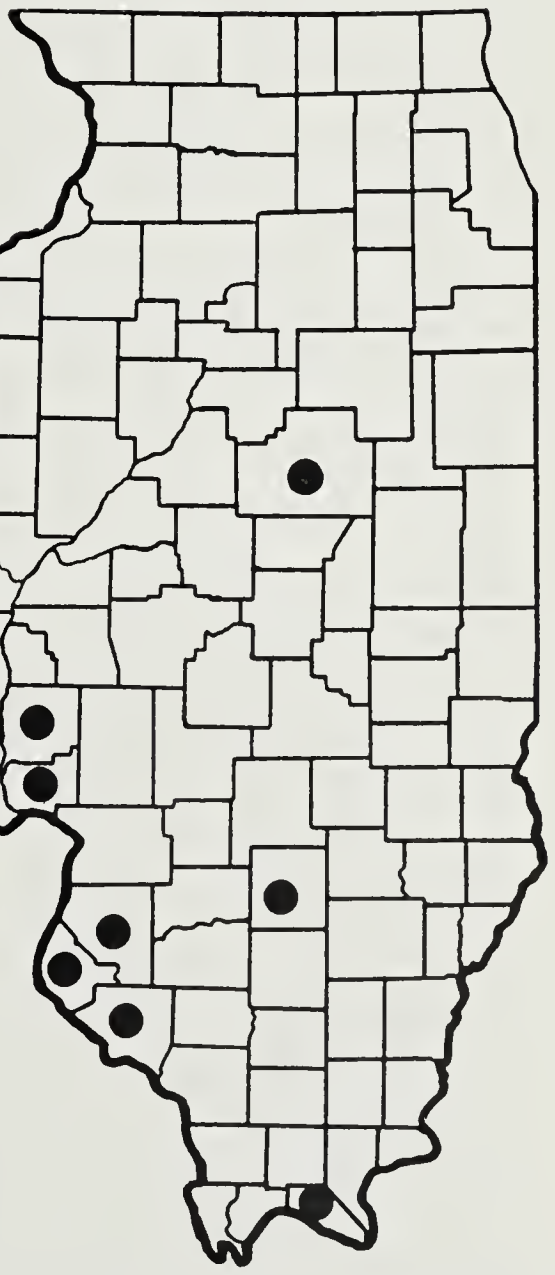

FIGURE 5. Distribution of *Vernonia baldwinii* in Illinois.

able variation. The five species named by Daniels are local races of the species and do not maintain themselves, forming a cline from one taxon to another. They are, therefore, placed in synonomy by this author.

4. VERNONIA FASCICULATA Michx. Fl. Bor. Am. 2:94. 1803.

Cacalia fasciculata (Michx.) Kuntze, Rev. Gen. 970. 1891.

Vernonia fasciculata Michx. var. *nebraskensis* Gleason, Bull Torrey Club 46:247. 1919.

Erect perennial herbs; stems 0.5-2.0 m tall, branched above, leafy, glabrous, often purple; leaves linear-lanceolate to oblong-lanceolate, 8-15 cm long, 0.5-3.0 cm wide, long-acuminate to acute, narrowed to base, denticulate to sharply serrate, glabrous above, glabrous or minutely puberulent beneath; inflorescence dense, hemispherical or flattened, 4-10 cm wide; heads crowded, sessile or short-peduncled, 18- to 21-flowered; flowers purple; involucre narrowly campanulate to subevlindrical, 6 mm high; phyllary tips rounded to subacute, appressed, imbricate, glabrous or puberulent, sparsely ciliate; pappus tawny to purple, inner bristles 7-8 mm long, outer bristles similar but shorter; achenes 3 mm long, puberulent on ribs, resinous in furrows; $n = 17$.

Vernonia fasciculata is found in rich low ground and prairies from Ohio to North Dakota, south to Oklahoma. It is distributed nearly throughout Illinois (Fig. 6).

5. VERNONIA ALTISSIMA Nutt. Gen. 2:134. 1818.

Vernonia albiflora Raf. Herb. Raf. 29. 1833.

Vernonia fasciculata Michx. var. γ T. G. Fl. N. Am. 2:58. 1841.

Vernonia fasciculata Michx. var. δ T. & G. Fl. N. Am. 2:58. 1841.

Vernonia gigantea Britton, Bull. Torrey Club 20:485. 1893 (in part).

Vernonia maxima Small, Bull. Torrey Club 27:280. 1900.

Vernonia gigantea Britton var. *pubescens* Morris, Proc. Biol. Soc. Wash. 13:179. 1900.

Vernonia maxima Small var. *pubescens* Morris, in Britton, Man. 919. 1901.

Vernonia altissima Nutt. var. *pubescens* (Morris) Daniels, Univ. Mo. Stud. Sci. 1:402. 1907.

Vernonia altissima Nutt. var. *taeniotricha* Blake, Rhodora 19:167. 1917.

Vernonia altissima Nutt. var. *brevipappa* Gleason, Bull. Torrey Club 46:248. 1919.

Vernonia altissima Nutt. var. *laxa* Gleason, Bull. Torrey Club 46:248. 1919.

Erect perennial herbs; stems 1-3 m tall, branched above, leafy, glabrous or subglabrous; leaves lanceolate to ovate-lanceolate, 15-25 cm long, 3-8 cm wide, long-acuminate, gradually attenuate to base, subentire to sharply and irregularly serrate, thin, glabrous or subglabrous above, pubescence on lower surface of short, straight, conic hairs, sometimes tomentellous along veins; inflorescence flattened or concave, 1-5 dm wide, open; heads (13-) 20- to 30-flowered; flowers purple; involucre campanulate, 4-6 mm high; phyllary tips rounded, rarely apiculate, appressed, imbricate, glabrous or puberulent, entire or sparsely ciliolate; pappus tawny to purple, inner bristles 5-7 mm long, outer bristles broader and shorter; achenes 3 mm long, puberulent, rarely sparsely resinous in furrows; $n = 17$.

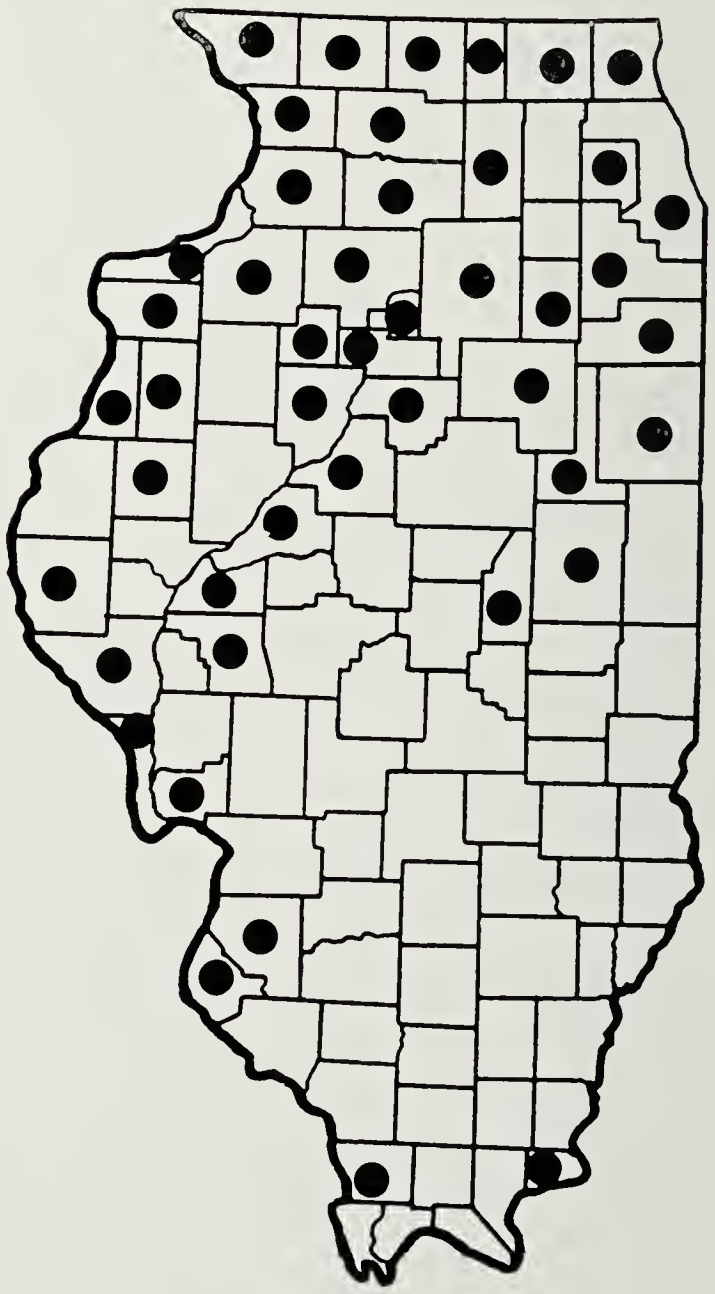

FIGURE 6. Distribution of *Vernonia fasciculata* in Illinois.

Vernonia altissima is found in low rich ground and open woods from New York, west to Missouri, south to Louisiana, and east to Georgia. It is frequent throughout Illinois (Fig. 7). Variation in *V. altissima* is also great. Pubescence of the lower leaf surface is primarily that of short, conic hairs, but long, multicellular hairs may be found in various degrees on the midrib and veins. Also the head size is variable and the inflorescence, normally open, will in some specimens tend to be compact. This has been suggested by some to be due to hybridization with *V. missurica* f. *missurica*, but the author considers this just variation within the species.

2. ELEPHANTOPUS L. Sp. Pl. 814. 1753.

Erect perennial herbs; stems leafy or scapiform, branching, pubescent; leaves alternate, simple; inflorescence corymbiform; heads discoid, 1- to several-flowered, subtended by sessile, triangular-ovate bracts; involucre urceolate, composed of 8 bracts, outer 4 shorter than inner 4; recepticle flat or nearly so; corolla unequally 5-cleft, blue; anthers sagittate, syngenesious; style-branches attenuate, minutely hispidulous outside; pappus of 5-8 short, flattened scales prolonged at the summit (in our species) into straight terminal bristles; achenes 10-ribbed.

Elephantopus is represented in Illinois by the following single species.

1. ELEPHANTOPUS CAROLINIANUS Willd. Sp. Pl. 3:2390. 1804.

Elephantopus violaceus Schultz-Bip. Linnaea 20:517. 1804.

Erect perennial herbs; stems 3-8 dm tall, branched in upper half, leafy, densely hirsute below, pilose or becoming glabrate above; leaves elliptic to obovate, 10-20 cm long, 3-10 cm wide, subacute to obtuse, abruptly narrowed or tapering to a winged petiole, crenate,

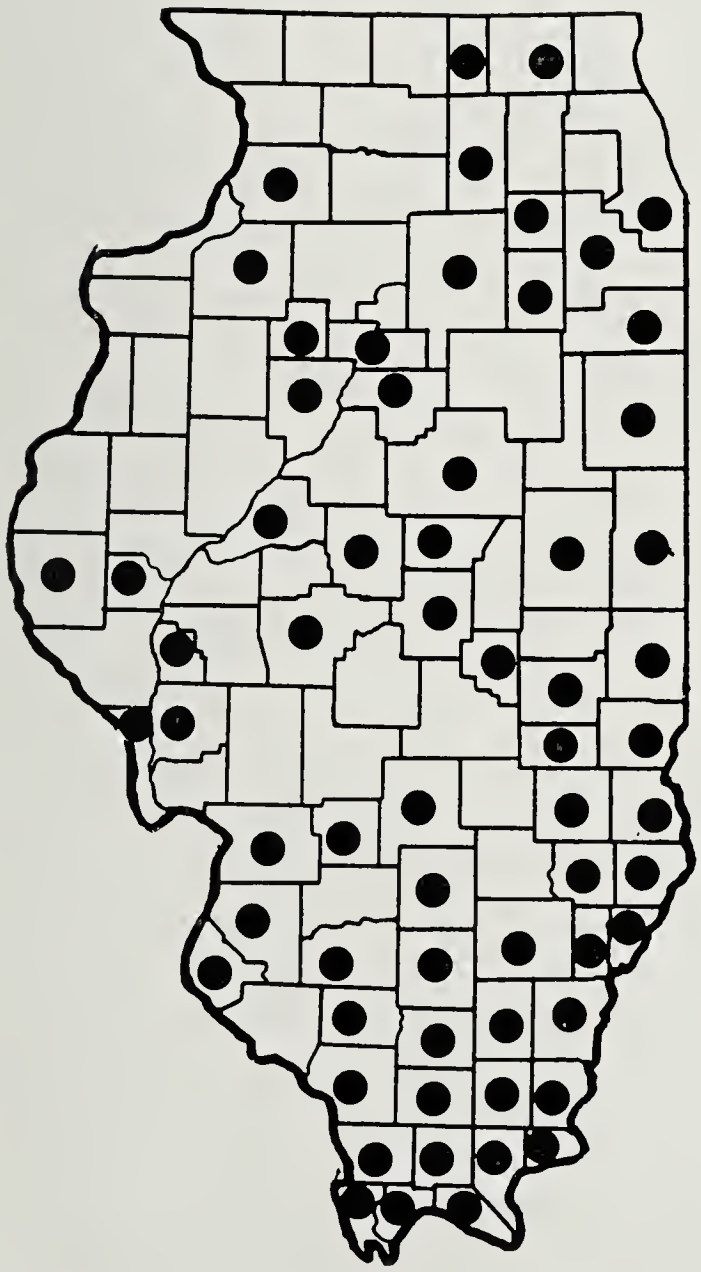

FIGURE 7. Distribution of *Vernonia altissima* in Illinois.

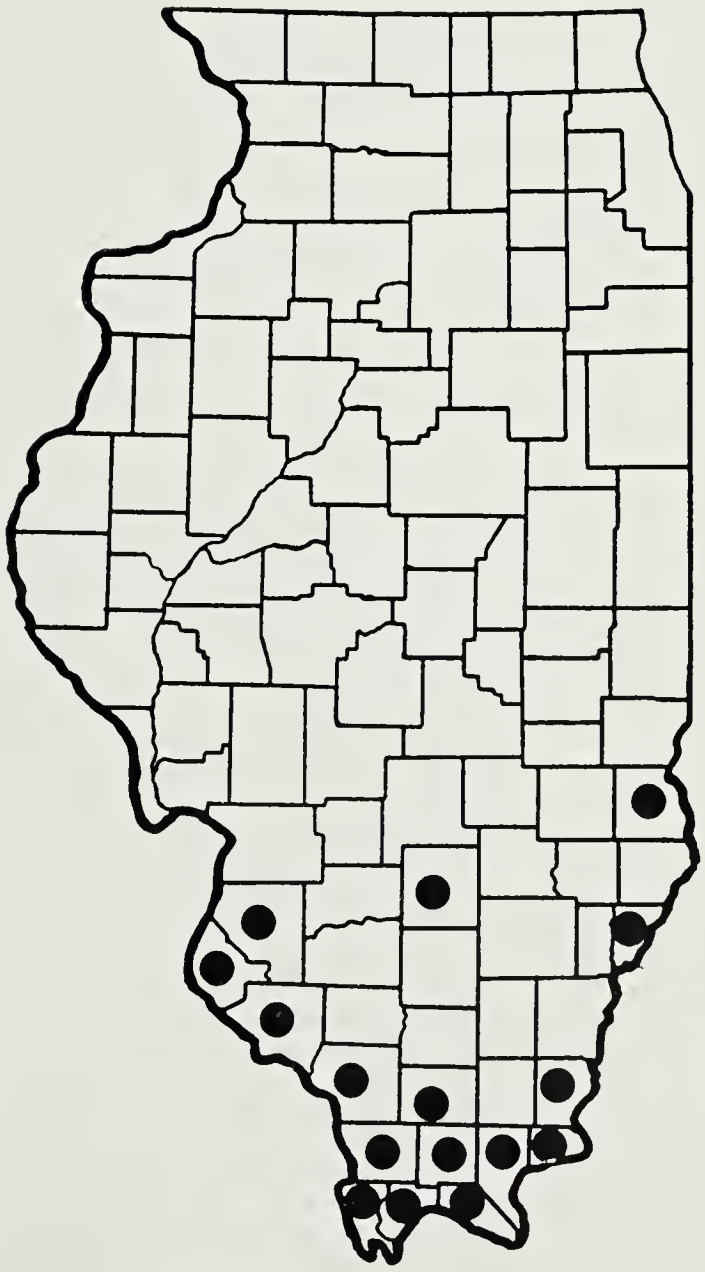

FIGURE 8. Distribution of *Elephantopus carolinianus* in Illinois.

papillose-pilosulous to subglabrous above, resinous-dotted and pilosulous beneath; heads discoid, 1- to 5-flowered, in numerous glomerules, subtended by sessile, triangular-ovate, foliaceous bracts; flowers blue; involucre about 8 mm high, composed of 8 bracts, outer 4 shorter than inner 4; phyllaries acuminate, thinly strigose and resinous, green with hyaline margins; pappus composed of 5-8 capillary bristles, 6 mm long, dilated at base, purplish; achenes 4 mm long, hispid; $n = 11$.

Elephantopus carolinianus is found in dry woods from New Jersey to Kansas, south to Texas, east to Florida, and also in Cuba and Puerto Rico. It is frequent in the southern third of Illinois (Fig. 8).

Acknowledgments

The distributional maps are based on specimens examined from the herbarium of Eastern Illinois University, the Field Museum of Natural History, the Illinois Natural History Survey, the Illinois State Museum, the Missouri Botanical Garden, Southern Illinois University, the University of Illinois, and Western Illinois University.

Manuscript received June 28, 1967.

POPULATIONS OF SMALL MAMMALS ON RAILROAD RIGHT-OF-WAY IN PRAIRIE OF CENTRAL ILLINOIS

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ABSTRACT. — Snap-trapping (approximately 1700 trap nights, 34 lines) was performed from July 1964 - July 1966, in tall grass prairie of railroad rights-of-way in central Illinois. The habitats are described generally, in regard to climate, soil, and vegetation. Small mammals (326) representing six species (*Mus musculus*, *Blarina brevicauda*, *Microtus ochrogaster*, *Peromyscus maniculatus*, *P. leucopus*, and one specimen of *Spermophilus tridecemlineatus*) were trapped. Other mammals observed were *S. franklinii*, *Marmota monax*, *Sylvilagus floridanus*, *Didelphis marsupialis*, *Mephitis mephitis*, and one *Mustela erminea*. *P. leucopus*, the most abundant mammal, was taken in all habitats of the study area. Sex and age ratios for *P. leucopus* were obtained. Its mean number of embryos was 4.28 ± 0.95 (2-6, N = 31). Its breeding subsides in winter and summer. Males of juveniles or adults generally exceed in number females of comparable age. Females nearing term of pregnancy are significantly less abundant than those with tiny embryos. Reproductive data on other small mammals are presented. *P. maniculatus* was usually associated with horsetails. *Mus musculus* was seldom taken in tall grass prairie, but was locally abundant on two occasions.

The "flat lands" of central Illinois have been disturbed drastically by agricultural practices. The deep, rich soil and level terrain of the "prairie peninsula" (Transeau, 1905) once supported deciduous red oak-maple-basswood-elm forest and tall grass prairie (Shelford, 1963; Gleason, 1912; Vestal, 1913; Sperry, 1935; Vestal and Heermans, 1945). Along the rights-of-way of several railroads in this area there is relatively undisturbed tall grass prairie, maintained by drainage, occasional clearing of brush, and burning. Hardly a "wilderness," this habitat seldom has been investigated systematically, even though it now provides the best source of information available on the former prairie in this intensively cultivated area. Although my departure from central Illinois precluded much of the intended investigation of this habitat, it is my purpose to record my findings especially in regard to the small mammals, so that this information may be available for future work.

METHODS

Small mammals were sampled by snap-trapping for 18 successive months, except September, December, and January. One preliminary trap line was set in July, 1964, and the remainder (33 lines) from February 22, 1965, to July 30, 1966. Although 50 traps were used in each line, in some warmer months large ground squirrels, grasshoppers, wind-blown leaves, and rain sprung some traps, and a vandal cut the treadle-locking wires from 20 traps on the next to the last line set out. With rolled cats used as bait, traps were placed at 10-yard intervals, usually in a straight line about 490 yards in length.

Observations on vegetation, road kills, and live animals provided some information, such as on the emergence of ground squirrels from hibernation. However, a systematic natural history

was not attempted. Home range was not determined.

The *Peromyscus* were assigned to age categories, defined as "adults" and "subadults" if characterized by reddish or dull brownish (fresh) dorsal pelages, respectively, and as "juveniles" if gray. Size and reproductive activity were utilized also in determining maturity. Immature *P. leucopus* fall in the range of variation for *P. maniculatus*. Owing to the far greater abundance of *leucopus* all juvenile *Peromyscus* were assigned to that species. The key characters listed by Hoffmeister and Mohr (1957) usually identify adults of these species. One valuable new diagnostic character utilized is the shape of the tail. In subadult *leucopus* it is short, but more attenuate distally than in *maniculatus*. All females were dissected in order to determine pregnancies. The crown-rump length was measured of the largest embryo in each pregnant mouse.

Study Area

Railroad rights-of-way were investigated in central Illinois, along a west-east line three miles east of Monticello through Champaign-Urbana to five miles east of St. Joseph—approximately 40 miles, including some areas (e. g., towns) not studied because of drastic disturbance. The railways were of the Illinois Central Railroad, west of Champaign, and the Norfolk and Western Railway, east of Urbana. The habitats along the rights-of-way varied markedly in width. In many places the boundaries of the habitat were delimited sharply by plowed fields or mowed roadsides.

Some Physical Factors: Climatic data obtained during the study are summarized in Table I. Graphs (not published) based on this table reveal slight differences between the two winters, in regard to temperature. December and January in 1964-1965 were cooler and warmer, respectively, than these months were in 1965-1966. Precipitation was low in the fall of 1964, compared to that of 1965. It was very low in October-January of 1965-1966, and there was little snow cover then. The total snowfall for November-March, 1964-1965, was significantly greater than for the same period in 1965-1966. The relative humidity changed little throughout the study.

The soil is sandy loam, blackish when moist, gray when dry, occasionally brownish. The roadbed of gravel and cinders supports some vegetation on the slopes, and contains burrows of several mammals (e.g., rabbit, ground squirrel, woodchuck) and some insects (e.g., ants). The water table is rather high, especially in a rainy spring. Extensive burning of the habitat was observed in May, 1966.

Vegetation: The vegetation was fairly homogeneous for many miles along the railroads. Vegetation types observed were (1) brush communities of blackberry (*Rubus*), wild plum and cherry (*Prunus*), and sumac (*Rhus*); (2) herbaceous communities dominated by tall grasses or forbs, of which goldenrod (*Solidago*) and milkweed (*Asclepias*) were conspicuous; (3) wetlands supporting cattails (*Typha*), willow (*Salix*), and sedges (*Carex*); and (4) dense stands of horsetails (*Equisetum*) intermixed with blackberry on dry sandy roadbed or bordering marshes. In the spring, annual plants push up through the broken down vegetation of the previous year, and may attain heights of six feet or more by autumn. Some introduced (or weedy) species were present such as dandelion (*Taraxacum*), red clover (*Trifolium*), alfalfa (*Medicago*), and mullein (*Verbascum*).

Mammal Populations

Carnivores were not common. Skunks (*Mephitis mephitis*) were found occasionally, dead on the highway adjacent to the railroad right-of-way. Domestic cats (*Felis catus*) were seen occasionally, in day or night. A lactating ermine (*Mustela erminea*) was found dead on the road four miles west of Bondville, adjacent to the study habitat. (This record is marginal for the known geographic range of the species.) Chicken bones were at the entrance of a den (fox?), but no hairs or scats were observed. The badger (*Taxidea taxus*) has been taken at St. Joseph, but none was seen in the area.

Other probable predators were *Spermophilus franklinii* and *Blarina brevicauda*. Both are well-known

TABLE 1.—General climatic data in the period July, 1964-July, 1966, from central Illinois.

	Temperature (°F)						Relative Humidity		Precipitation (inches)	Total Snowfall (inches)
	Means			Extremes						
	Max.	Min.	Mean	Max.	Min.	Max.	Min.	Mean		
July 1964.....	85.6	65.8	75.7	94	52	100	29	75.0	2.41	0
Aug. 1964.....	84.5	60.9	72.7	101	51	100	31	71.0	2.59	0
Sept. 1964.....	78.3	55.8	67.1	93	41	99	19	68.0	2.18	0
Oct. 1964.....	65.4	40.7	53.1	82	30	98	20	61.0	0.16	0
Nov. 1964.....	55.4	35.1	45.3	77	6	98	21	67.0	3.42	6.9
Dec. 1964.....	35.5	22.9	29.2	53	2	96	48	84.0	1.79	3.6
Jan. 1965.....	36.7	18.5	27.6	62	−5	95	33	75.0	4.13	8.3
Feb. 1965.....	39.2	20.5	29.9	60	−6	.....	.....	.....	1.46	5.7
March 1965.....	38.6	24.0	31.3	59	10	93	33	69.0	2.50	10.6
April 1965.....	62.6	43.2	52.9	84	30	100	34	73.0	5.64	0
May 1965.....	79.1	56.9	68.0	89	39	100	29	71.5	3.66	0
June 1965.....	81.5	60.7	71.1	91	52	98	28	70.5	3.05	0
July 1965.....	83.4	63.5	73.5	94	53	100	20	73.5	5.12	0
Aug. 1965.....	81.1	61.5	71.3	92	46	100	35	74.8	6.89	0
Sept. 1965.....	76.6	57.1	66.9	89	39	100	33	77.0	5.91	0
Oct. 1965.....	65.0	44.8	54.9	82	28	97	23	66.0	1.96	0
Nov. 1965.....	53.6	35.1	44.4	71	17	99	27	68.0	1.24	0
Dec. 1965.....	44.3	30.6	37.5	63	15	97	35	74.0	2.88	1.3
Jan. 1966.....	29.7	14.0	21.9	55	−12	93	27	66.0	0.40	0.5
Feb. 1966.....	37.5	21.3	29.4	58	2	94	22	67.5	2.07	6.6
March 1966.....	51.0	32.2	41.6	73	16	97	22	66.0	1.91	3.1
April 1966.....	58.3	40.1	49.2	76	29	100	24	67.0	4.64	0.1
April 1966.....	58.3	40.1	49.2	76	29	100	24	67.0	4.64	0.1
May 1966.....	68.5	46.4	57.2	87	30	100	21	70.5	2.99	0
June 1966.....	84.1	60.3	72.2	95	49	100	45	77.0	3.02	0
July 1966.....	90.2	67.1	78.7	101	56	100	35	79.5	1.34	0

omnivores. Franklin's ground squirrels were observed from May to July. Five adults were seen along one trapline on May 4, 1966, in prairie at Mayview. Others were seen nearby, so that a "colony" apparently was established. Dens and runways were abundant in the tall grass. In 1964 and 1965, this ground squirrel was not seen in late summer or fall. On May 4, 1966, one was seen eating a road-killed pheasant.

Two opossums (*Didelphis marsupialis*) were found dead on the road at Mayview prairie. Two large herbivores observed were the woodchuck (*Marmota monax*), and the cottontail rabbit (*Sylvilagus floridanus*). A juvenile woodchuck was observed June 4-5, 1966, near Mayview, Illinois. Only two adults were seen in this study, both in the morning, in May, 1966.

A thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) was caught in the area, and the species was fairly abundant locally along adjacent roadsides. However, its habitat was short (mowed) grass, and it was rarely observed in the

tall grasses of the study habitat. The one specimen obtained was an adult male taken July 29, 1965, one mile north of Allerton Park, Monticello. The small testes had apparently regressed in size. Thirteen-lined ground squirrels emerged about April 4, 1965, and March 26, 1966, at nearby Lake of the Woods (Mahomet, Illinois) where the species is abundant. In 1965, two were first seen on April 7 on short grass roadside near Mayview prairie.

Occasionally, fox squirrels (*Sciurus niger*) were seen between Urbana and St. Joseph (only gray squirrels were seen in Champaign-Urbana). A pregnant fox squirrel was found dead on the road April 7, 1965. It contained three embryos, 26 mm. in crown-rump length.

The small mammals trapped were the white-footed mouse, *Peromyscus leucopus*; the deer mouse, *P. maniculatus*; the feral house mouse, *Mus musculus*; the short-tailed shrew, *Blarina brevicauda*; and the prairie vole, *Microtus ochrogaster*. The white-footed mouse, also abundant in nearby woodlands, was the most abundant species, slightly exceeding 70 per cent of the small mammals trapped.

The age and sex composition of the monthly samples of *P. leucopus* are shown in Table II. Adults and sub-

TABLE 2.—Reproductive data for *Peromyscus leucopus* from mammals caught in 27 traplines (45-50 traps each). September, December, and January data are not available.

	Feb.	March	April	May	June	July	Aug.	Oct.	Nov.
No. and percent pregnant									
1965	0 0%	14 45%	6 67%	5 100%	1 50%	0 0%	2 67%	1 25%	0 0%
1966	0 0%	.....	.....	.....	2 100%	0 0%	.....	.....	.....
No. formerly pregnant (lactating)									
1965	0	0	0	1	1	1	1	1	1
1966	0	0	1	0	4	.....	.....	.....	.....
Some males with swollen scrota									
1965-1966	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Mean litter size									
1965-1966	.....	4.14	4.86	4.60	3.7	.....	4.00	4.00	.....
Sex ratio (adults and subadults) male:female									
1965	5:3	33:30	31:10	4:6	5:4	10:4	6:3	10:8	0:4
1966	9:4	.....	0:1	.....	3:7	3:1	.....	.....	.....
Fraction Juveniles									
1965	1/9	0/63	9/47	6/16	2/11	0/14	1/10	7/25	1/5
1966	0/13	.....	.....	5/5 (after fire)	2/12	0/4	.....	.....	.....

adults usually numbered more than the juveniles, especially in winter and summer, but less so in spring and fall. Assuming an approximately even sex ratio at birth (Svendson, 1964; and others), the ratio of males to females increases significantly (0.05 level) in juveniles (21:9), and is significantly greater (0.02 level) in adults and subadults (119:85).

Reproductive data are summarized in Table II. There is little breeding in late fall and winter. Another pause in breeding activity occurs in summer, as reported for white-footed mice of Michigan woodland (Burt, 1940). Embryo counts averaged 4.28 (2-6; N, 31), with mode of four and standard deviation of the mean of 0.95. From this, the population range can be predicted as 1-7, which is in agreement with the data of Burt (1940), Svendson (1964), and others. There is no statistical evidence for change in litter size through the year.

Thirty-nine embryos of nine pregnant *P. leucopus* were distributed randomly in either the left or right cornu of the uterus. The number of embryos for the left was 20, right 19. The modal distribution was two on each side, closely approached by two on one side and three on the other.

In 31 pregnancies, most females of *P. leucopus* had small embryos.

Classes	*Pregnancies*	*Embryos*
1-4 mm.	11	45
5-8 mm.	6	30
9-12 mm.	7	32
13-16 mm.	2	9
17-20 mm.	5	18

The sum of the pregnancies in classes one and two (17) is significantly greater (0.05 level) than that of four and five (7). Birth may have lowered the frequencies somewhat of observed pregnancies in the late development class(es). However, data of Svendson (1964) indicate that at birth foetuses are longer than 20 mm., even if his "body length" exceeds crown-rump length. The evidence indicates that mice in early pregnancy are more susceptible to trapping than other pregnant mice. If the frequencies for crown-rump length are all increased to 11 (the frequency of the largest class and the lowest crown-rump length), then the number of pregnant females would be increased by 24, and the ratio of adult and subadult males to females would become 119:109. This hypothetical ratio is not significantly different from a 1:1 ratio at the 0.05 level. However, the significant preponderance of juvenile males over juvenile females (see above) indicates a skewed sex ratio prior to the age of breeding, in this habitat. Susceptibility to trapping of females in early pregnancy needs further investigation.

P. leucopus was taken in abundance in all habitats of the study area. Although it is often said that peak populations of rodents occur in autumn, and that they are low in winter, I did not find this true (Table II).

Twenty-five *P. maniculatus* were taken in this study, usually in horsetails. Three were taken ¼ mile from them in May, 1966, immediately subsequent to burning of the dead prairie grasses. The burning, incidentally, revealed surprising numbers of

mouse runways among the charred grass roots and stems and on the bare ground. Females of several species were more common then than males, and the juveniles (*Peromyscus* sp.) were more common than adults the month following the burning (but the data are scanty). The ratio of males to females for *P. maniculatus* was 10:15, not significantly different ($P<0.5$). The deer mouse was taken March-June, 1965, and February-May, 1966. Two embryo counts of three and five were observed in June and February, respectively. Three of three females were lactating on May 4, 1966; they contained no embryos.

Only nine *Microtus ochrogaster* were taken in this study. They were usually juvenile. Two adults, neither trapped although two traps were located less than three feet away, had a nest of dried grasses under a large piece of pressed wood (February, 1966). In the nest were 13 pits of wild plum, all of which had been opened by gnawing, by the *Microtus*, on one flat side. A runway led about 2½ feet from the nest (about 8 inches from the board) to a hole, at the entrance of which was a gnawed, partially exposed root. The nest was abandoned by March.

Nineteen *Blarina brevicauda* were caught; the catches were small (13) and distributed throughout the year. A female taken April 2, 1966, had five embryos, 6 mm. in crown-rump length. A small (subadult) specimen was taken October 5, 1965.

Thirty-one feral *Mus musculus* were taken, mostly adults. The ratio of males to females was observed as 16:11, the numbers not significantly different. House mice were taken in winter and spring of 1965 and not taken thereafter until October and February of 1965-1966. Thus, in the grassland feral house mice were locally abundant at certain times for reasons unknown. On February 22, 1965, two were pregnant (6,5 embryos).

Acknowledgments

The field work was done while the author was on the faculty of the University of Illinois. Theodore Frank, an Honors student, helped with the field work in 1965. Carlos Pinkham, a gradnate student, helped in 1966. Professor Ferron Andersen formerly of the Illinois School of Veterinary Medicine but presently of the Department of Zoology, Brigham Young University, provided the climatological data in Table I. Professors Horace A. Hays and Theodore M. Sperry, of Kansas State College of Pittsburg, and Charley M. White, of Wisconsin State University, Stevens Point, offered appreciated criticisms of the manuscript.

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Manuscript received Aug. 7, 1967.

A DEVELOPMENTAL STUDY OF ROOTS OF PRESUMED SEED FERN ORIGIN FROM THE UPPER PENNSYLVANIAN OF ILLINOIS

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ABSTRACT.—A coal ball petrification of Upper Pennsylvanian age has been found containing extensive remains of roots of a new type including two root apical meristems and a bud-like meristem. Ontogenetic sequences of root development are described and illustrated. The root meristems have large, multicellular, initial zones producing root cap and meristematic tissues which subsequently give rise to differentiating cells of the protoderm, ground meristem, and procambium. Serial sections of the roots illustrate a development of the vascular cambium much like that found in the roots of extant, dicotyledonous plants. A comparison of the present specimens with similar root types from the Pennsylvanian is made. In general features of organization the material described in this account appears most like roots of medullosan seed ferns.

The present study describes some exceptionally well preserved roots of probable seed fern origin. Included in the study is a description of root apices and early ontogeny of the primary and secondary bodies. No previous reports of comparable material have described the ontogeny of the root or any apical meristematic regions. The few available descriptions of roots found in coal balls and having only primary tissues, such as that of Hoskins (1931), do not describe ontogenetic development. The present description is based on a coal ball petrification from the Berryville locality in which there occurred three axes that had the apical meristems present. Two of these axes are typical roots which lie adjacent to one another and are attached to a portion of a branching root system (Fig. 14). One root apex having a triarch stele has been sectioned transversely (Figs. 7-13). The other root having a pentarch stele was of sufficient length so that the apical initial zone could be studied from longitudinal sections and the basal portion studied from transverse sections (Figs. 1-6). The third axis is a problematical bud-like organ with vascularized "scale-leaves" (Figs. 15-23). The bud-like structure is attached to an axis which has typical root anatomy and lies adjacent to the better preserved system. There is, however, no cellular connection to the better preserved roots. The bud-bearing axis can be identified with the root system for reasons discussed later in the text.

MATERIAL AND TECHNIQUE

The location from which the specimen was collected is near the town of Berryville, Illinois (Section 7, T 2 N, R 13 W, Sumner Quadrangle, Lawrence County, Illinois). The most recent stratigraphic position and age published is that of Taylor (1965) which identifies the coal balls as coming from the Calhoun Coal, Mattoon Formation, McLeansboro Group. The age is Upper Pennsylvanian.

Serial sections for the study were made by employing the cellulose peel technique as described by Joy, Willis, and Lacey (1956). The coal ball bears the designation U. Ia. C. B. 315.

Description

Organization of the Root Apex. In longitudinal section the growing tip of the root has a cellular arrangement that is typical of a multicellular apical meristem as opposed to that commonly found in meristems having a single apical cell (Fig. 1). At the extreme apex of the root the pattern of cell arrangement lacks specific organization into zones or histogens. The promeristem of the root apex consists of two poorly defined zones of small, isodiametric cells of relatively uniform appearance (Fig. 1, indicated by PI). Two areas of promeristem at the root tip suggest that the root was in the early stages of undergoing a dichotomy. In the upper portion of Figure 1, a peripheral ground meristem zone is present (indicated by GM) which surrounds a central area of procambium (indicated by PC). As one proceeds toward the tip of the root, axial rows of cells converge on each of the two initial zones which are separated by a zone of larger cells, presumably representing ground meristem. These axial rows of meristematic cells are not traceable to specific initials because the cells of the initial zone are non-aligned. The alignment of cells occurring back of the initial zone indicates the later occurrence of periclinal divisions in cells produced from the promeristem initials. In general, such a row of cells extends only a short distance before irregularity disrupts the sequence. These aligned meristematic cells give rise to the differentiated cells of the protoderm, the ground meristem, and the procambium. The root cap results from divisions of the promeristem initials.

The apical meristem studied in transverse section shows promeristem cells (Fig. 7, indicated by PI) that are apparently differentiating into a root cap through increased size and elongation. The root cap is bluntly cone-shaped (Figs. 7 and 8, indicated by RC).

Cells of the protoderm occur on the flanks of the initial area in the specimen studied in transverse sections. These cells are contiguous with those of the root cap and form a recognizable small-celled protoderm one cell layer in thickness (Fig. 8, indicated by PD). Stages in epidermal differentiation are precluded by the poor preservation of the apical meristem sectioned longitudinally. The outer portion of this specimen consists of dark-colored, poorly preserved remains of the root cap and protoderm (Fig. 1).

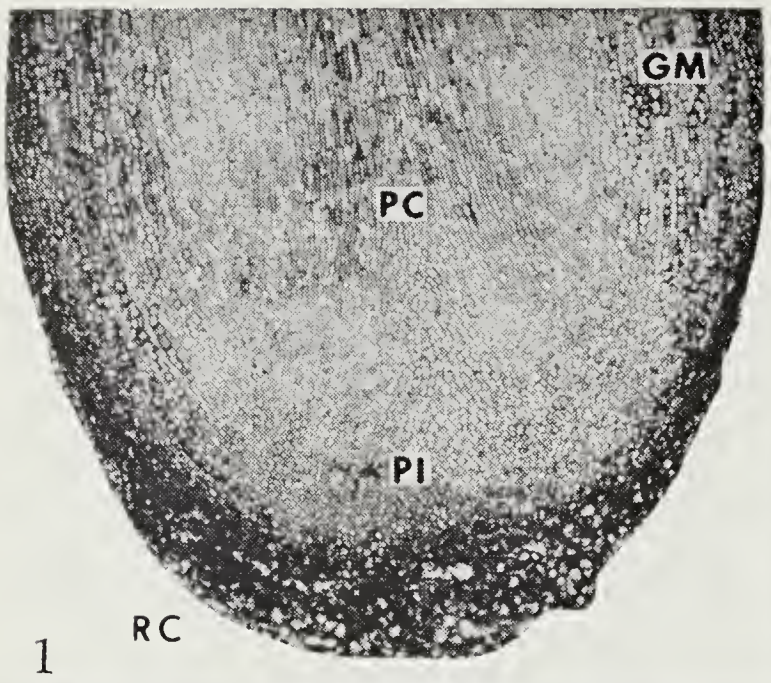
Cells of the ground meristem appear as typical parenchyma cells except that they are aligned vertically in short rows, and apparently represent daughter cells produced by successive anticlinal divisions. These cells are characterized by their large diameters, isodiametric to slightly vertically elongate shape, and compact disposition within the tissue (Fig. 1).

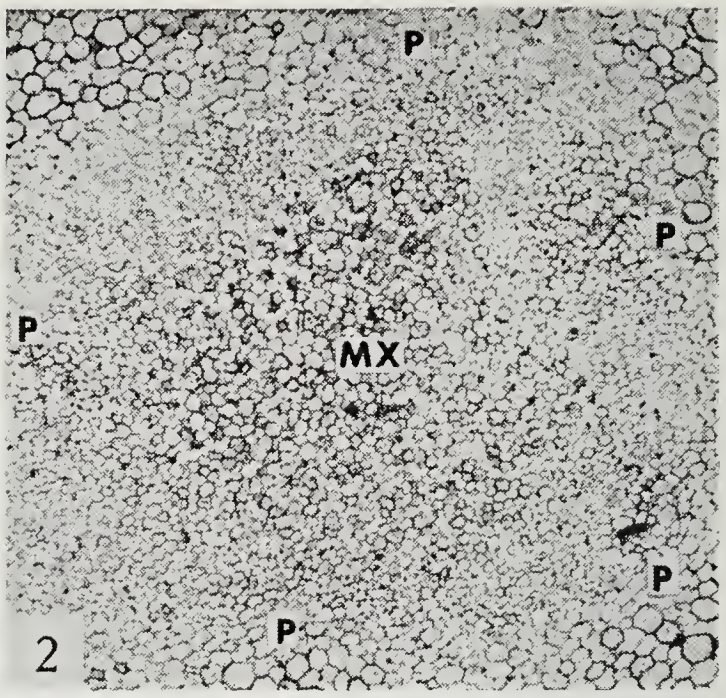
In transverse sections of the root apex, the first evidence of vascular tissue differentiation consists of the appearance of a central procambial core made up of extremely narrow cells. It is of interest to note that the first indication of vascular tissue differentiation occurs in the center of the procambium in a region that is ultimately destined to form the central portion of the metaxylem of the stele. The procambial cells retain narrow diameters, whereas cells in the developing cortex increase markedly in diameter (Fig. 8).

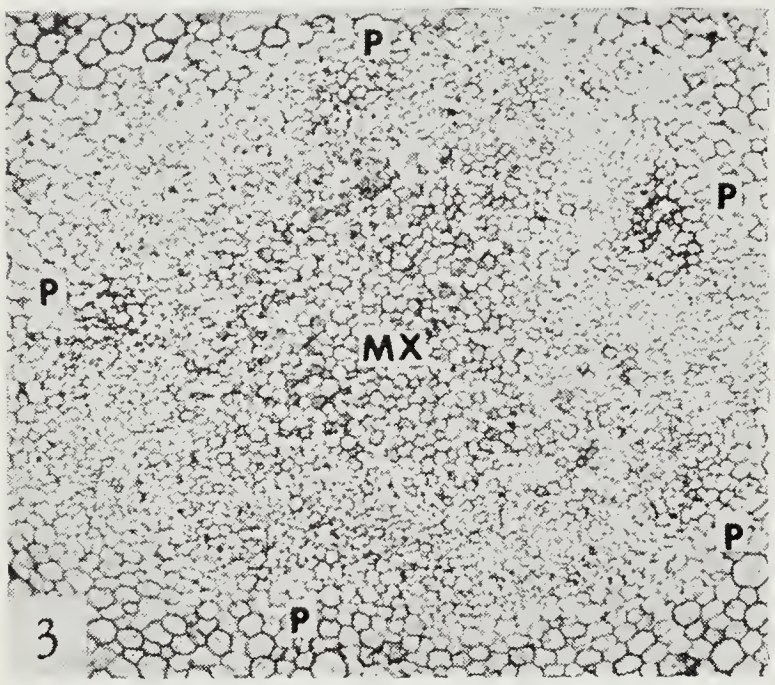
In the region of initial differentiation of vascular tissue procambial cells enter a phase of elongation during which they do not increase greatly in diameter. This elongation phase occurs simultaneously with the initiation of secondary wall thickenings which consist of narrow, simple, scalariform bars.

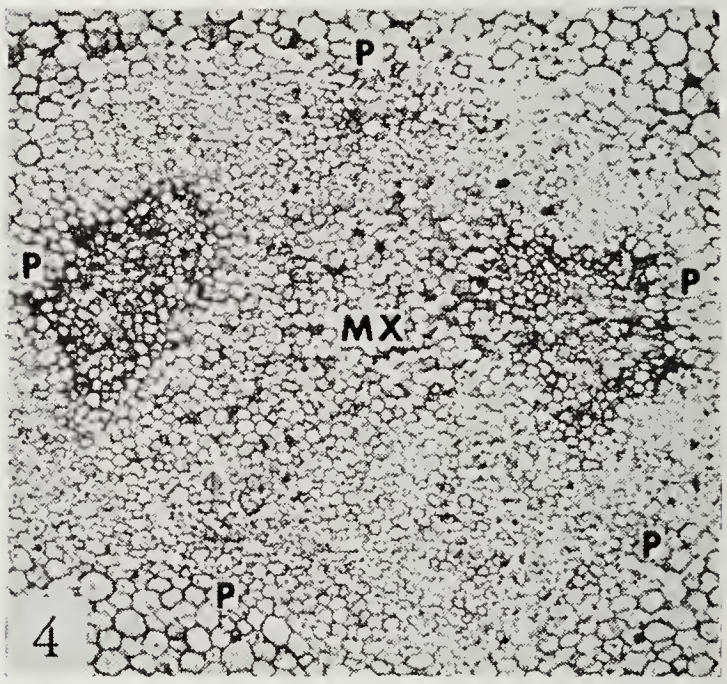
Phloem can be distinguished in these roots as relatively large cells with dark walls. The first indication of the presence of phloem in the region behind the promeristem occurs at the periphery of the provascular tissue (Fig. 2). Two of the five phloem groups in Figure 2 are more prominent than the other three, however, this may be the result of crushing of the tissue. By comparing the two largest phloem areas in Figures 3 and 4, one can determine that phloem development is centripetal.

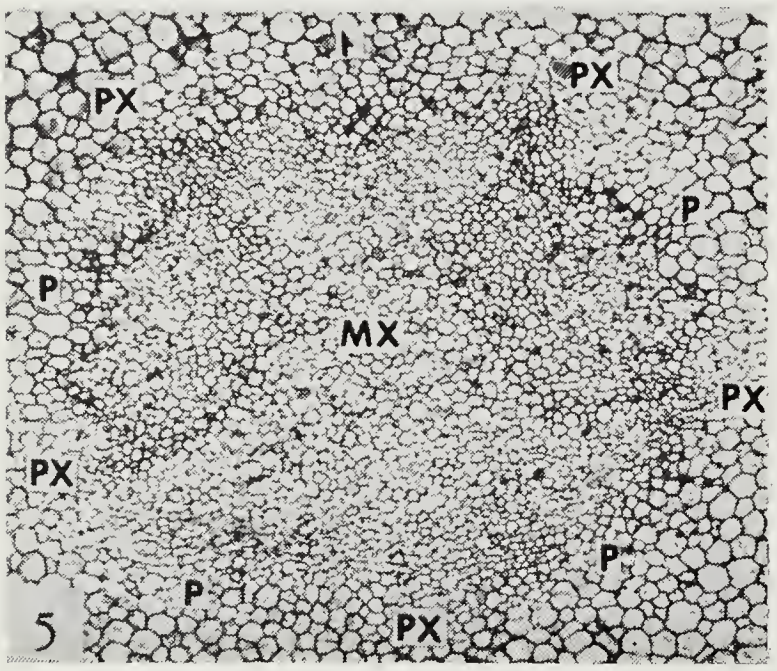
The initial appearance of protoxylem consists of several (five in the instance illustrated) clusters of small-diametered cells located midway between the phloem positions on the periphery of the provascular cylinder. These areas are identified (Figs. 5 and 6, indicated by PX) by the relatively narrow diameters of the cells, topographic posi-











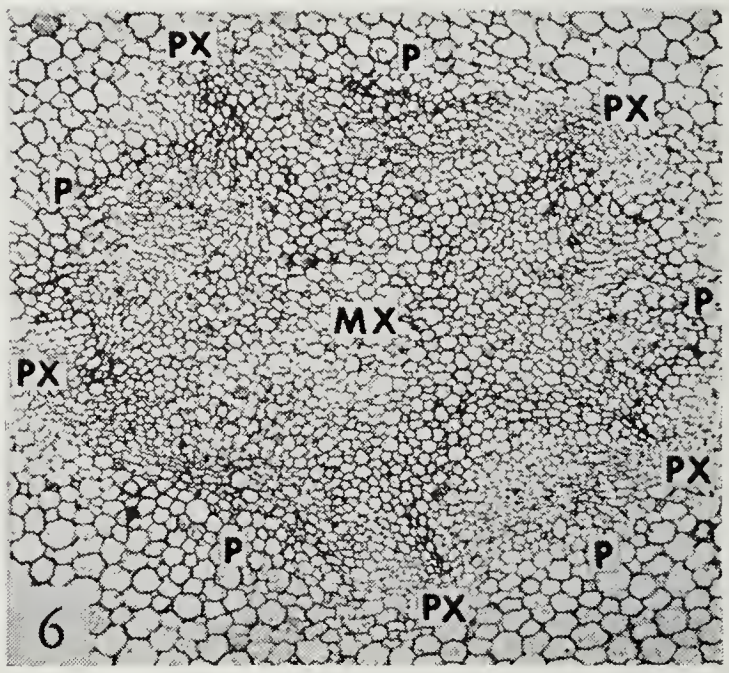


PLATE I

FIGURE 1. Median longitudinal section of apex of a root having a pentarch stele. Promeristem initials are indicated by PI, procambium by PC, ground meristem by GM, and root cap by RC. U. Ia. c. b. 315 B_2(Bottom)#4, section 14. X 18.

FIGURES 2-6. Representative transverse sections of a pentarch root illustrating successive stages in the maturation of the primary stelar tissues. Figure 2. A level just behind the growing tip where areas of phloem first become apparent. U. Ia. c. b. 315 B_2(Bottom)#225. Figure 3. A level at which the characteristic dark-walled cells of the phloem are more obvious. U. Ia. c. b. 315 B_2(Bottom)#210. Figure 4. A level illustrating the progressive centripetal maturation of phloem in two of the better preserved phloem regions. U. Ia. c. b. 315 B_2(Bottom)#170. Figure 5. A level at which the regions of protoxylem first become obviously apparent. U. Ia. c. b. 315 B_2(Bottom)#130. Figure 6. A level showing the mature primary condition of the stele. U. Ia. c. b 315 B_2(Bottom)#51. In these figures primary phloem is indicated by P, metaxylem by MX, protoxylem by PX. All figures X 39.

tion, and relatively thick walls which have apparently resulted from more rapid secondary wall deposition. Protoxylem cells do not undergo any marked increase in diameter beyond this level in the root, whereas metaxylem tracheids continue to increase in diameter. It is this ontogenetic sequence that produces the characteristic configuration of the primary stele, which may be pentarch as seen in Figure 6, or triarch as seen in Figure 9.

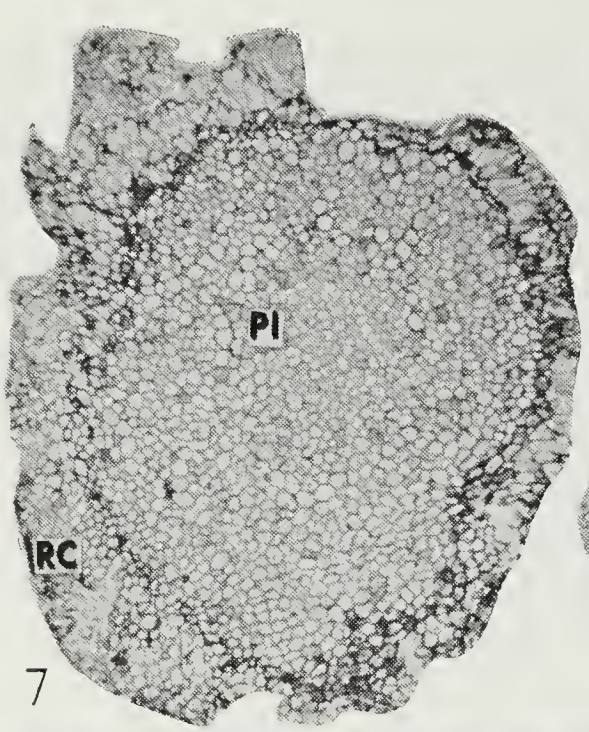
The Mature Primary Condition. The disposition of the primary tissues resulting from the organization of the apical meristem is typical of that found in several types of seed fern roots. The central part of the stele consists of metaxylem tracheids with occasional strands of parenchyma. No pith is present. Metaxylem tracheids measure 33μ in diameter and are characterized by simple scalariform thickenings on the walls (Fig. 26). In a few instances, these pits appear to be bordered as in Figure 25.

An examination of all the roots shows the primary xylem to be triarch (Fig. 9), tetrarch (Figs. 14 and 24), or pentarch (Fig. 6). Protoxylem and metaxylem cells intergrade with regard to diameter so that the transition between these tissues is gradual. Protoxylem tracheids may be identified by their position, smaller diameter, and presence of widely spaced secondary thickenings on the walls.

Two types of cells have been distinguished in the primary phloem. Typical protophloem elements possess thin walls, tapering ends, and diameters of about 43μ (Fig. 26, indicated by PP). A centripetally developed zone about three to twelve cells wide apparently represents sieve cells of the metaphloem. These cells possess extremely dark walls and are axially elongate. Cells of this type measure about 60μ in diameter and are indicated by MP in Figure 26.

At levels in the root which apparently represent the mature primary condition, no evidence of a pericycle was observed. The cortex consists of a homogeneous parenchyma composed of isodiametric cells which apparently lack intercellular spaces. The epidermis is typically not preserved, however, at some levels remnants of this tissue were identified.

Secondary Development. Initial stages of secondary tissue development are present in the roots. A complete sequence is presented for a triarch root in Figures 10-13. This includes stages from the first appearance of secondary tracheids to a condition in which radial rows with numerous tracheids exists. The cambium arises in the zone between the metaxylem and the phloem in areas about midway between the protoxylem points. The initial stage of cambial production is shown in Figure 10 at the indication C. The production of secondary tissue occurs in this specimen a short distance behind the level of differentiation of the primary vascular tissues. The most prominent portion of the secondary vascular tissue consists of radially oriented rows of secondary tracheids which develop between the protoxylem points (Figs. 10-13). Initially, the cambium is difficult to distinguish, however, as secondary development continues, it becomes more evident (compare Figs. 10 and 11 with Figs. 12 and 13). As already noted, the cambium initially develops midway between the protoxylem points and subsequently develops laterally toward the protoxylem areas. No cambium develops opposite the protoxylem areas, and consequently, a continuous ring of cambium is never produced in the roots. Parenchyma cells occupy the areas opposite the protoxylem points and effectively divide the secondary wood into segments that correspond in number to the protoxylem points. The vascular



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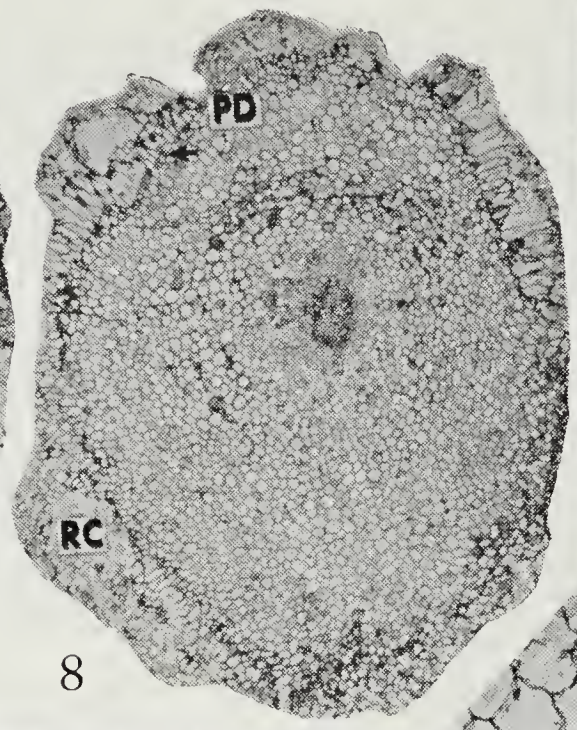

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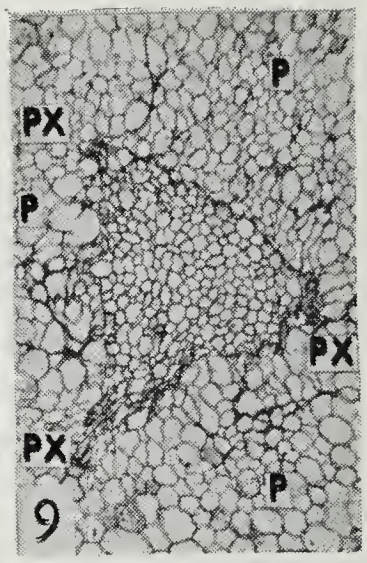

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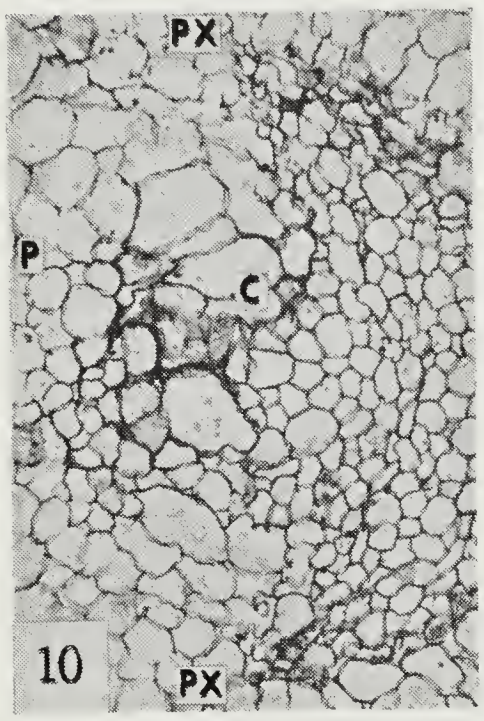

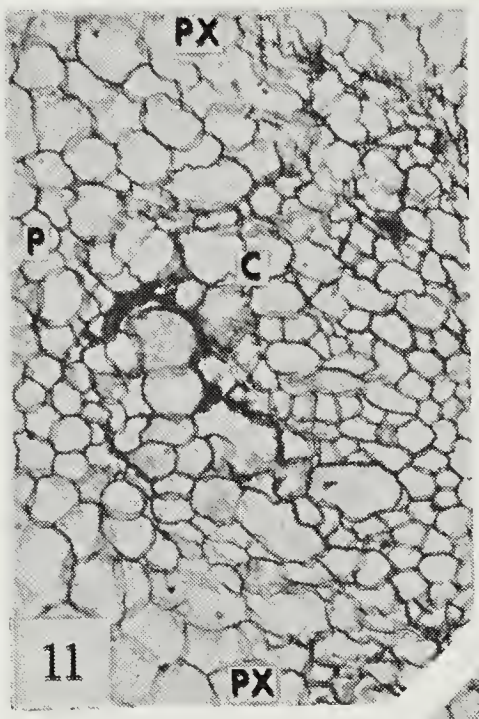

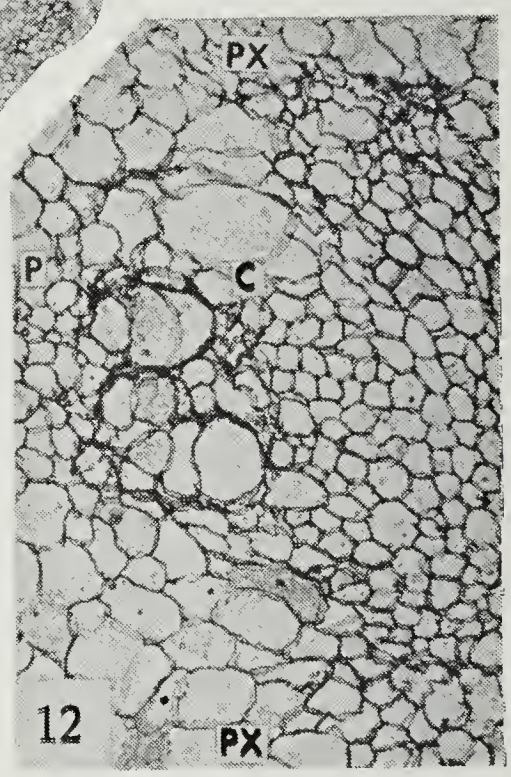

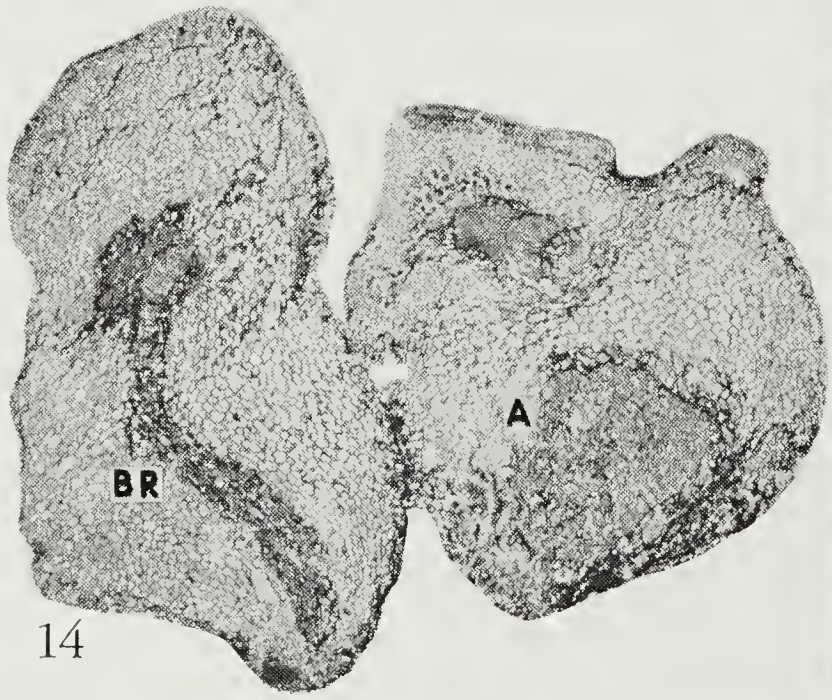

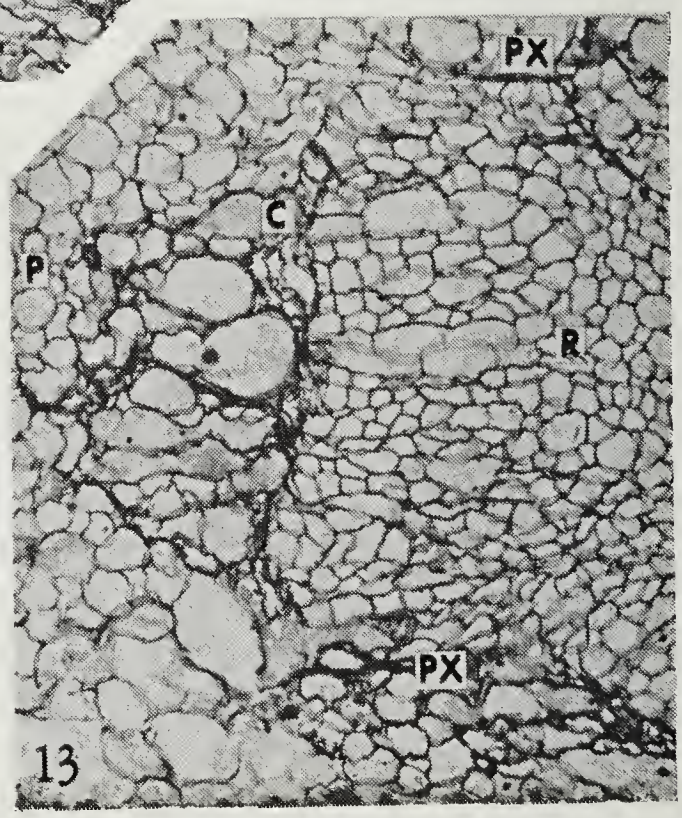

PLATE II

FIGURE 7. Transverse section of the root cap and promeristem initial zone of a root having a triarch stele. Promeristem initials indicated by PI., root cap by RC. U. Ia. c. b. 315 B_2(Bottom) #71. X 18.

FIGURE 8. Transverse section of the root cap, protoderm, and procambial region of the same root as that shown in Figure 7. Root cap indicated by RC, protodermn by PD. U. Ia. c. b. 315 B_2(Bottom)#50. X 12.

FIGURE 9. Mature primary condition of the triarch stele of the same root as that shown in Figures 7 and 8. Protoxylem areas indicated by PX, primary phloem areas by P. U. Ia. c. b. 315 B_2(Bottom)#39. X 49.

FIGURES 10-13. Transverse sections at progressively lower levels of the portion of the stele toward the left in Figure 9. The figures illustrate successive stages in cambial development and production of secondary vascular tissues. Figure 10. A level showing the region in which the vascular cambium is initiated. U. Ia. c. b. 315 B_2(Bottom)#35. Figure 11. A level illustrating an early stage in secondary xylem production. U. Ia. c. b. 315 B_2(Bottom)#26. Figure 12. A level illustrating an increased amount of secondary xylem and the crushed cells of the vascular cambium. U. Ia. c. b. 315 B_2(Bottom)#21. Figure 13. A level illustrating radial files of tracheids and vascular rays of the secondary xylem zone. U. Ia. c. b. 315 C(Top)#5. In the figures protoxylem areas are indicated by PX, phloem by P, vascular cambial zones by C, wood or vascular ray by R. All figures X 83.

FIGURE 14. Transverse section of a portion of the root system illustrating several tetrarch roots. A branch root is indicated by BR. A branch root with anomalous stelar tissues is indicated by A. U. Ia. c. b. 315 C(Top)#10. X 6.

cambium produced comparatively little secondary phloem; in some instances radially aligned rows of three to six large sieve cells have been found (Fig. 24 at indication P). Pitting of the secondary tracheids does not differ from that of the metaxylem elements and is of the uniseriate scalariform type. Rays are relatively infrequent and are usually uniseriate or biseriate (Fig. 13 indicated by R.) Steles of a few representative roots with abundant secondary tissues are shown in Figures 14 and 24.

Branching of the roots is very common. Branch roots arise opposite a protoxylem point of the parent root (Fig. 14 indicated by BR). Apparently, the branch roots did not arise endogenously as occurs in a typical seed plant because no evidence of a separate cortex to the branch roots could be found. In one instance anomalous structure has been found in one of the branch roots (Fig. 14 at the indication A) which contains tracheids that twist around one another at all angles. The aberrant tracheidal mass is in turn surrounded by phloem and cortex. Periderm has not been observed.

A *Bud-bearing Axis*. One axis has been discovered which terminates in a bud-like apex. Behind this apical region, where mature tissues are present, the axis enlarges and is typically root-like in morphology. Figures 15-21 illustrate progressively lower levels of the bud-like portion of the axis. The transition from the bud-like area to that at which typical root structure is present occurs in a poorly preserved portion of the specimen which has been sectioned very obliquely so that structural details of the transitional phase cannot be determined. Figure 22 shows the base of the bud-like area in attachment to the root portion of the specimen, while Figure 23 illustrates the lowest level of the specimen at which typical root structure is present.

The most striking feature of the apical portion of the bud is the presence of an armor of small, scale-like, lateral appendages which cover the growing tip of the axis (Figs. 15-18). These lateral appendages exhibit several features which are characteristic of leaves, but their exact morphological nature is not certain. For the sake of convenience, these lateral appendages will be referred to as scale-leaves in the following portions of the text. The scale-leaf primordia are apparently produced from superficial tissues of the apical meristem as are typical leaf primordia. At a level such as that shown in Figure 17, the scale-leaves appear to be arranged in a spiral pattern. The cells forming the apical meristem possess thin walls, a homogeneous appearance, and are much smaller than those of the surrounding scale-leaves. Since the meristem was studied only from cross sections, it was not possible to determine whether there was a single apical initial or a group of initials in the promeristem region.

Although, superficially, the scale-leaves appear to be borne in a fairly uniform spiral arrangement, no consistent phyllotaxy can be determined. In all, the bud-like portion bears twenty-four scale-leaves which range up to 3 mm in width. Epidermal cells of the scale-leaves are poorly preserved. Rela-

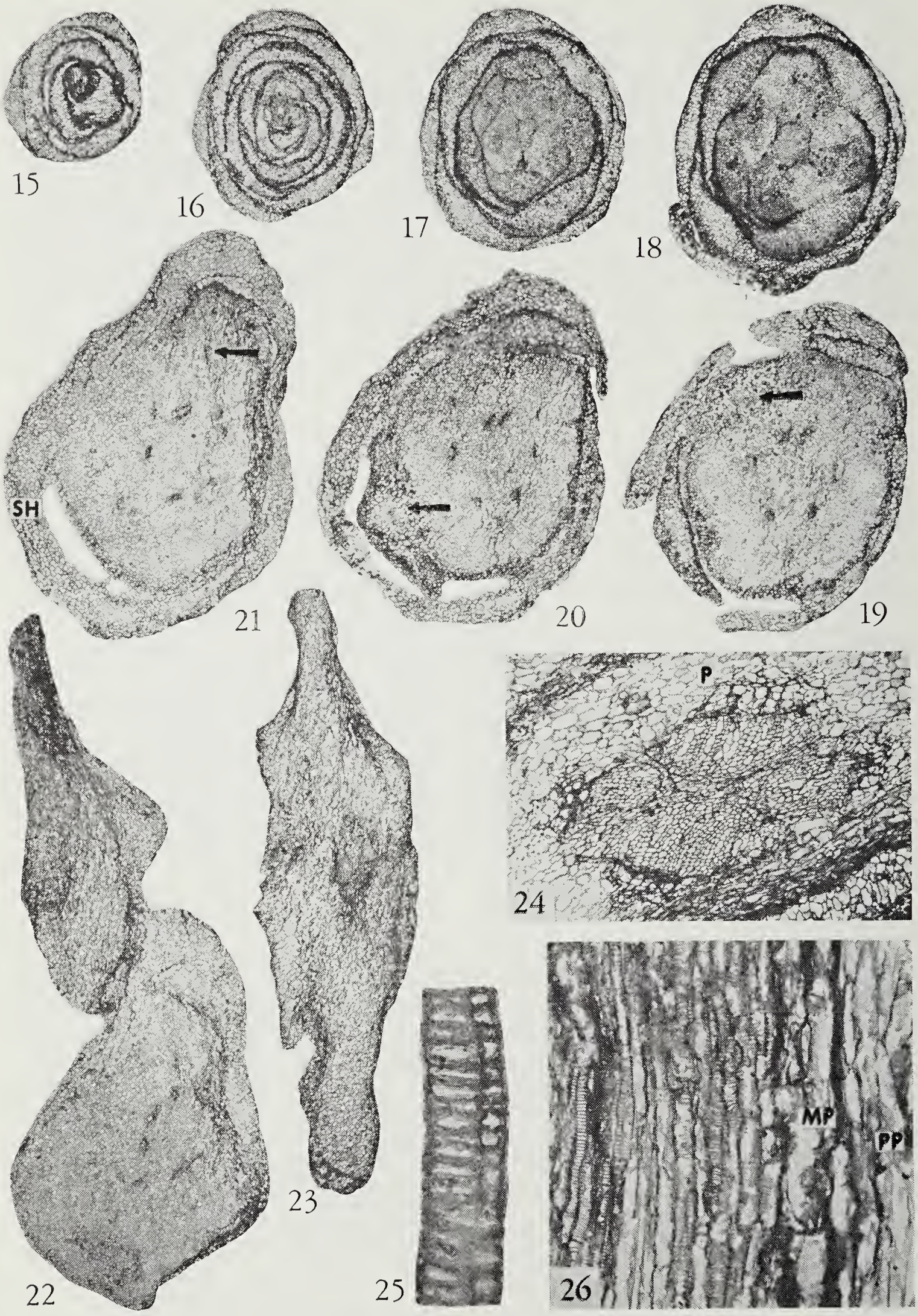

PLATE III

FIGURES 15-21. Representative series of transverse sections at progressively lower levels through a bud-like portion of the axis. Figure 15. Most distal level

illustrating the overlapping scale-leaves which overtop the axis. U. Ia. c. b. 315 B_2(Bottom)#32. Figure 16. A level at the very summit of the growing point where a few cells of promeristem are surrounded by a broad zone of scale-leaves. U. Ia. c. b. 315 B_2(Bottom)#18. Figure 17. A level through the promeristem region showing scale-leaf primordia and the bases of more mature scale-leaves. U. Ia. c. b. 315 B_2(Bottom)#1. Figure 18. A level showing a central region of presumed procambial tissue, vascular traces in the bases of several scale-leaves, and the distal-most part of the sheath. U. Ia. c. b. 315 B_2(Bottom)#4a. Figure 19. A level showing the lower portions of a series of vascular strands to scale-leaves arranged in a ring. Arrow indicates a vascular strand in the base of a scale-leaf. Several fragmentary areas of sheath partially surround the axis and scale-leaves. U. Ia. c. b. 315 C(Top)#1. Figure 20. A level where the sheath forms a continuous zone around the axis, and the bases of the two lowermost scale-leaves are present. Arrow indicates a vascular strand in the base of a scale-leaf. U. Ia. c. b. 315 C(Top)#16. Figure 21. A level showing the tissues of the sheath continuous with those of the axis and the base of the lowest scale-leaf. The arrow indicates a vascular trace entering the base of the lowest scale-leaf. U. Ia. c. b. 315 C(Top)#39. All figures X 9.

Figure 22. Transverse section through the base of the bud-like portion shown in Figures 15 through 22 where it is in attachment to the portion of the specimen with root anatomy. U. Ia. c. b. 315 C(Top)#56. X 8.

Figure 23. Oblique transverse section through the portion of the specimen shown in Figures 15-22 where typical root anatomy is present. U. Ia. c. b. 315 C(Top)#88. X 7.

Figure 24. Transverse section of a representative tetrarch stele with secondary vascular tissues. An area of primary and secondary phloem is indicated by P. U. Ia. c. b. 315 B_1(Bottom)#8. X 14.

Figure 25. Longitudinal view of a metaxylem tracheid showing uniseriate, scalariform, wall thickenings. The junction of two walls is shown. U. Ia. c. b. 315 C(Top)#45. X 330.

Figure 26. Longitudinal section of a portion of a stele showing the primary vascular tissues. The tissues are, from right to left; protophloem indicated by PP, metaphloem indicated by MP, and xylem. U. Ia. c. b. 315 B_1(Bottom)#18. X 75

tively large diameter parenchyma cells form the ground tissue of the scale-leaves. In the central portion of the lamina of the scale-leaves this zone is about eight cells wide and tapers to one cell row at each lateral edge. A single vascular trace enters the base of each of the more mature scale-leaves (Figs. 19-21). Leaf traces are present only in the basal regions and do not extend into the free laminate portion. The scale-leaves are broadly attached to the parent axis.

The first indication of the production of a scale-leaf is the departure of a trace from the stele of the parent axis. This trace angles outward slowly traversing the cortical zone until it occupies a position about twelve cell layers beneath the epidermis. An outward bulging of the epidermal tissue of the main axis delimits the base of the scale-leaf. Complete separation takes place along this line by the progressive development of two equal, lateral invaginations. Unlike the remaining scale-leaves of the specimen, the lowest scale-leaf is not symmetrical at its base and has a free lamina extending along only one of its sides. Distally, each scale-leaf decreases in thickness, becomes laminate or flattened, and gradually tapers. The terminal portions of the scale-leaves tightly enclose and overarch the growing tip of the parent axis (Figs. 15 and 16).

The major features of the vascular system of the bud-like portion of the specimen are as follows. Ten vascular strands to scale-leaves have been traced back toward their points of origin from the vascular system of the main axis. The vascular trace of each scale-leaf arches inward across the cortex and fuses with a vascular trace from a lower scale-leaf. At lower levels these fused vascular strands come to assume positions somewhat equidistant from each

other forming a ring around the center of the main axis. The fusion of vascular strands continues until the trace from the lowest scale-leaf joins the system. This latter event occurs at a level where the specimen is sectioned very obliquely and preservation is poor. At this level, however, there appears to be four main vascular strands. At this same level, the bud-like portion of the specimen is attached to a root-like region whose long axis lies nearly at right angles to the bud-like portion (Fig. 22). The preservation of the root portion of the specimen improves sufficiently to determine that it has a tetrarch stele exhibiting the same structure as the roots previously described (Fig. 23). Due to the preservation of the specimen, it is impossible to determine whether the bud-like portion represents a lateral of the root-like part or is merely a continuation of the axis.

A curious feature of the specimen is the presence of a sheath of large-celled parenchyma which covers the lower portion of the bud. At its lowest level the sheath is continuous and encloses the bases of the lowest scale-leaves (Fig. 21, indicated by SH). Toward the apex the sheath becomes broken into fragments and is sloughed away completely so that the scale-leaves make up the outer part of the upper region of the bud.

Discussion

Several accounts on the structure of roots having a similar stelar anatomy to those described here have been published. These have been, for the most part, roots attached to, or in association with, stems of the seed fern genus *Medullosa*. Although similar in structure, the roots described in this paper differ in several respects from those previously described.

Although the roots of *M. anglica* as described by Scott (1899) show numerous points of similarity to those described here, several important structural details representing distinct differences deserve consideration. Roots of *M. anglica* possess protoxylem elements with spiral secondary wall thickenings, metaxylem tracheids with numerous rows of crowded bordered pits, secondary tracheids with rows of crowded bordered pits present only on the radial walls, and primary xylem in a triarch configuration. Periderm development apprently occurred early in ontogency in the roots of *M. anglica*. These features of the roots of *M. anglica* were substantiated by Arber (1903) who also described the phloem.

Roots thought to be borne by species of *Medullosa,* described by Hoskins (1931), differed from those of *M. anglica* in that some of the older roots lacked periderm. In addition, note, the primary xylem was found to be triarch, tetarch, or pentarch.

Steidtmann (1944) described roots in attachment to stems of *M. noei* which showed a similar range in the number of protoxylem points. In addition, the roots of *M. noei* were described as having spiral thickenings on the walls of the protoxylem tracheids and bordered pitting on the walls of the metaxylem tracheids. The pits of the secondary tracheids in both stem and roots is multiseriately arranged and bordered. As in *M. anglica,* roots of *M. noei* were found to have periderm at relatively early stages in root development.

In contrast to *M. Anglica* and *M. noei,* roots described in this study have simple scalariform thickening on both metaxylem and secondary tracheids. Protoxylem tracheids also have simple scalariform thickenings. Periderm has not been observed in any of the roots, however, these roots, in respect to the amount of secondary vascular tissues pres-

ent, correspond to developmental stages described for *M. anglica* and *M. noei*. Roots of *M. noei* and those described here have a similar range in the number of protoxylem areas. This feature is clearly one that cannot be relied upon in distinguishing taxa since the specific number of protoxylem points present in a given order of root is known to vary greatly in a single root system of numerous kinds of living vascular plants.

Roots of such monostelic genera of seed ferns as *Lyginopteris* and *Heterangium* differ markedly from those described in this paper and cannot be easily confused with them.

The preponderance of evidence suggests that the roots described here are most similar to those of *Medullosa*. They are clearly distinct, however, from all previously described roots assigned to that genus. Because of the great amount of variability demonstrated and small number of specimens available for examination, no new name is being assigned.

A significant feature of the material is the exceptionally complete sequence of early developmental stages present. Such stages of root development are extremely rare in the fossil record although they are known to occur (Daugherty 1963).

One of the most curious features of the material is the structure that has been referred to as a bud. This structure clearly represents either a lateral appendage or a continuation of an axis that was a root. Whether this bud represents an early stage in shoot development or merely some atypical portion of the root system cannot be determined. The presence of the lateral appendages referred to as scale-leaves seems to suggest that the bud represents an early stage in shoot development. Ontogenetically, these scale-leaves developed from superficial tissues at the apical meristem and are vascularized. These features are characteristic of true leaves. In contrast to these leaf-like features, no consistent phyllotactic pattern could be determined. Moreover, vascularization does not extend into the free portions of the appendages. These latter features are more typical of epidermal appendages. The occurrence of leaves on a root is not known to occur in any extant or fossil vascular plant of which the author is aware.

Acknowledgment

The author is indebted to Dr. Donald A. Eggert, Department of Botany, University of Iowa, for his able guidance and assistance during the preparation of the manuscript. This research was supported in part by National Science Foundation grants GB-1540 and GB-4126.

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Manuscript received August 3, 1967.

RAINFALL VARIABILITY RELATIONS ON SMALL AREAS

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ABSTRACT. — Data from three dense raingage networks operated on areas of 10, 100, and 550 square miles for periods of 7 to 11 years in Illinois were used to determine (1) the time and space variability of storm, monthly, and seasonal precipitation and (2) the relationship of precipitation relative variability to areal mean precipitation, maximum network precipitation, storm duration, precipitation type, synoptic weather type, and season. With storm rainfall, the strongest individual correlation was between relative variability (V) and mean precipitation (P). Seasonally, only small differences were found in the degree of correlation between V and P. V increased with increasing area and was substantially greater with unstable precipitation than with the steady types. Grouped according to synoptic weather type, the highest V was obtained with air mass storms and the lowest with low center passages and warm fronts. The relative variability of monthly precipitation maximized and minimized in different months of the year between networks, apparently reflecting climatic differences in the precipitation from central to southern Illinois. In general, the relative variability was lowest in winter and highest in the warm-season months, and months with below-normal precipitation tended to have higher relative variability than above-normal months. Results from this study should provide background information valuable in hydrometeorological applications and in verification programs for weather modification experiments.

The existence of relatively large spatial and temporal variability in precipitation, particularly in thunderstorms and rainshowers, is generally recognized among meteorologists and hydrologists. However, knowledge to define this variability in quantitative terms has been inadequate, especially with respect to small areas and various types of storm conditions. In hydrometeorology, such knowledge is pertinent to establishing watershed raingaging facilities and in interpreting rainfall-runoff relationships. In weather modification studies, precipitation variability must be considered in both the planning and verification of field experiments. Failure to do so may lead to poorly designed sampling programs and invalid interpretation or experimental results.

In Illinois, data were available from several concentrated raingage networks operating for periods ranging from 7 to 11 years and encompassing areas of 10, 100, and 550 square miles. These data have been used to determine quantitatively relative variability on a storm, monthly, and seasonal basis and to show the relationship of precipitation variability to areal mean precipitation, maximum network precipitation, storm duration, precipitation type, synoptic weather type, and season.

DATA AND ANALYTICAL PROCEDURES

The three sampling networks are shown in Figure 1. The Little Egypt Network in southern Illinois has been in operation for 9 years and consists

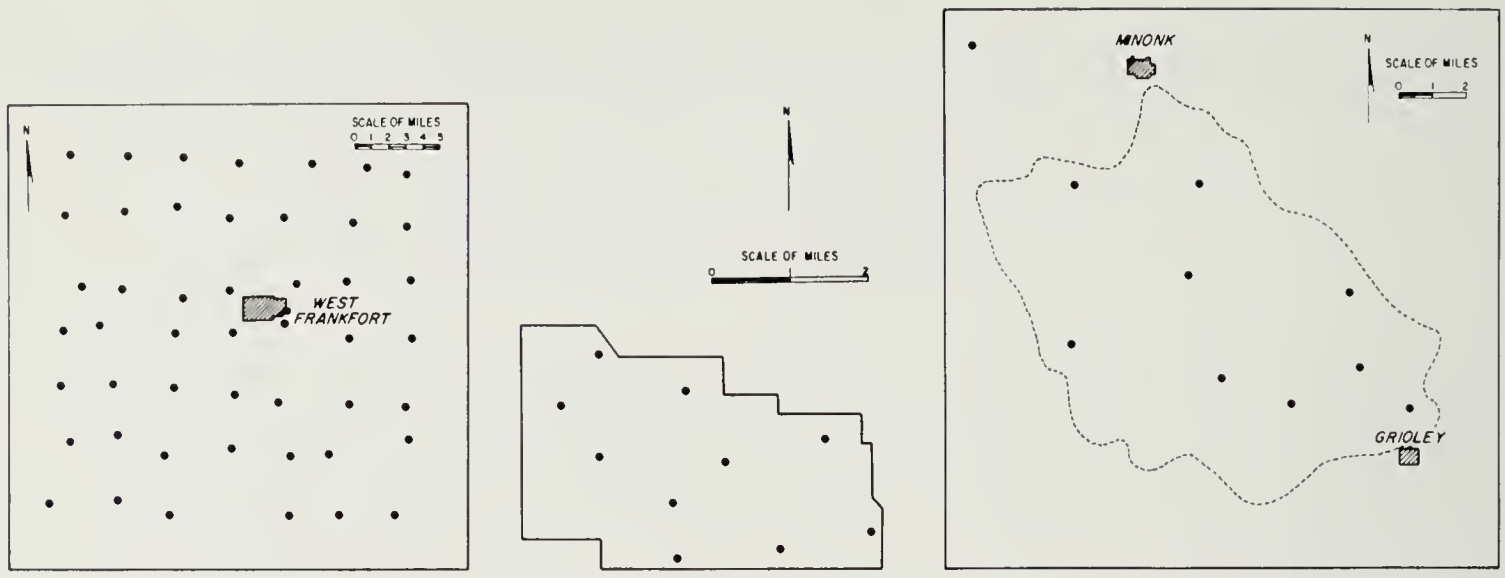

FIGURE 1. Raingage Networks Used in Study

of 49 raingages in 550 square miles. The 9-year sample (1958-1966) provided 964 storms in which the areal mean rainfall equalled or exceeded 0.01 inch, the basic requirement for inclusion in the variability study. The Panther Creek Network of 9 raingages on 100 square miles in north-central Illinois contributed 724 storms to the study during the 1954-1960 period. During 1954-1964, the Boneyard urban network of 10 raingages in 10 square miles, located at Champaign-Urbana in east-central Illinois, provided 1066 storms. All networks are located in relatively flat terrain which is characteristic of the major midwestern agricultural regions. For the few storm data that were missing because of occasional mechanical problems at some of the gages, amounts were estimated through the use of isohyetal maps.

The spatial relative variability was obtained by the simple method presented by Conrad and Pollak (1950), in which it is defined by:

$$V = 100(AD/M) \qquad (1)$$

where V is the relative variability in percent, M is the mean of the sample, and AD is the average deviation from the mean.

After determination of the mean rainfall, average precipitation duration, and average deviation in each storm, Equation (1) was used to determine the relative variability. For storms that did not have measurable rainfall at some gages in the network, a second calculation of relative variability was made after eliminating the zero or trace observations. This provided a more useful variability measure for some applications. At the same time, the number and percentage of gages (area) without precipitation were tabulated as additional statistics.

In the storm studies, correlation analyses were used to determine the degree of relationship between storm relative variability (V) and areal mean precipitation (P), maximum network precipitation (P_m), and storm duration (D), when the storms were grouped by synoptic weather type, precipitation type, and season. Simple and multiple correlation coefficients were determined with the data expressed in both linear and logarithmic terms. From the correlation analyses, decisions were made on the regression analyses to be performed with respect to data transformation and independent variables.

In the monthly and seasonal calculations of spatial variability in total precipitation (P_t), the relative variability was calculated with Equation (1). Relations were developed between V and P_t and comparison made of the magnitude of V between seasons and between wet and dry periods. Probability estimates of monthly variability were also developed.

STORM RELATIONS

In the study of individual storms, correlation analyses produced higher coefficients with logarithmic transformations than with the linear variables, as indicated by graphical plots of the data in preliminary studies. The differences are illustrated in Table 1 in which linear and logarithmic computations of correlation coefficients (r) and coefficients of determination (r^2) are shown for V, P, and D in summer storms on the Little Egypt Network.

Table 1 is typical of the results obtained with the data grouped according to season, precipitation type, and synoptic weather type. As indicated in Table 1, only a small improvement in the relationships was found when V was related to both P and D as opposed to P alone. This is due to the strong correlation between P and D; as mean rainfall increases the storm duration also tends to increase. Consequently, it was decided to develop regression equations relating the logarithms of V and P.

TABLE 1.—Linear and Logarithmic Correlations Between V, P, and D in Summer Storms on Little Egypt.

Transformation	r			r^2(%)		
	V-P	V-D	V-P, D	V-P	V-D	V-P, D
Linear	—0.47	—0.51	0.55	22	26	30
Logarithmic	—0.75	—0.70	0.79	56	49	62

Regression equations were developed with the data grouped according to season, precipitation type, and synoptic weather type. Further analyses indicated that the seasonal grouping primarily reflected the frequency distribution of precipitation types in the four seasons. Therefore, for evaluation purposes the seasonal grouping was eliminated and the precipitation type classification was selected and is summarized in Table 2.

TABLE 2.—Precipitation Type Comparisons of Relative Variability.

Precipitation Type	Number of Cases	Average Relative Variability (%) for Given Mean Rainfall (in.)					
		0.05	0.10	0.25	0.50	1.00	2.00
All Gages							
Little Egypt (550 Square Miles)							
TRW	436	117	85	56	41	30	23
RW	227	83	61	41	30	23	17
R	219	54	40	27	20	15	11
S	82	45	33	22	16	..	..
Panther Creek (100 Square Miles)							
TRW	146	72	55	38	29	22	17
RW	317	67	50	35	27	21	16
R	170	52	39	27	20	15	11
S	91	52	39	27	20	..	..

Boneyard (10 Square Miles)

TRW	442	63	44	28	20	14	10
RW	235	51	36	23	16	11	8
R	239	37	26	17	12	8	6
S	151	31	24	17	14	..	..

Only Gages with Measurable Amounts

Little Egypt

TRW	89	69	49	38	30	23
RW	71	54	38	29	22	17
R	44	35	25	19	14	11
S	35	27	19	15	..	..

Panther Creek

TRW	52	42	32	27	21	17
RW	47	38	29	24	20	16
R	40	31	23	18	14	11
S	40	31	23	18	..	..

Boneyard

TRW	49	36	24	18	13	10
RW	40	29	20	15	11	8
R	29	22	15	11	8	6
S	29	23	17	13	..	..

Precipitation Type Grouping

In Table 2, V is shown for selected values of mean rainfall on each of the three sampling networks based upon all sampling points and upon only those raingages with measurable precipitation. If more than one precipitation type occurred in a storm, it was assigned to the predominating type with respect to the total storm precipitation. Precipitation was classified as thunderstorms (TRW), rainshowers (RW), steady rain (R), and snow (S). Other types were too few to be included in the study.

Before discussing Table 2 further, it should be emphasized that snow measurements are subject to relatively large sampling errors, especially in strong winds and with low-density snow. Consequently, the values of V are probably larger than the true values in nature, and the snow relations should be considered only rough approximations for comparison purposes.

In general, Table 2 shows the expected trends for the relative variability to decrease with decreasing area and increasing mean rainfall, and to be substantially greater with the unstable types of precipitation (TRW, RW) than with the steady types (R, S). For the smaller mean rainfalls, which are more likely to result from storms of smaller areal extent and shorter duration than the heavy storms, a significant decrease in variability was found when only gages with measurable precipitation were used. However, the sampling area decreases in these cases and this contributes also to the decreasing trend in V.

The decreasing relative variability with increasing mean rainfall results from general trends for heavier storms to last longer and have greater areal extent. The increasing relative variability with increasing area results from the greater range of rainfall values which occur, on the average, as the

size of the sampling area increases. The primary importance of Table 2 with respect to these two relationships of relative variability is the establishment of quantitative measures of the V-differences with increasing area and volume of precipitation.

Another quantitative measure of equal or more interest in Table 2 is the V-differences among precipitation types. On the Little Egypt Network in southern Illinois, the ratios of the unstable rainfall types, TRW and RW, to the stable type, steady rain, were approximately 2.1 and 1.5, respectively, for all gages. On Panther Creek, these ratios were 1.4 and 1.3, respectively, and on the Boneyard Network they averaged 1.7 and 1.4. It is believed that the primary cause of the network ratio differences proceeding northward is the greater proportion of storm rainfall associated with air mass showers of relatively small areal extent and steep spatial gradients in the southern part of the state (Huff, 1966).

Snow values have been omitted for mean precipitation greater than 0.50 inch in Table 2 because of the small number of cases. The same curve fit both R and S on Panther Creek, whereas the relative variability of snow was somewhat less than steady rain on the other two networks. Considering areal size, the higher values of V for snow on Panther Creek compared with Little Egypt is unexpected. This apparent inconsistency is believed to arise from the occurrence of lower-density snow with stronger average winds on Panther Creek. These conditions resulted in greater drifting and less reliable sampling than on the Little Egypt network.

Synoptic Type Grouping

Table 3 summarizes the results of determining the V-P relation for all gage observations after grouping of the data according to synoptic weather type. This was done to obtain a quantitative measure of the effect of major storm types on V. Six basic types of storms were used, as shown in Table 3. Low centers and occluded fronts were combined, because the few cases of occluded fronts appeared similar in V characteristics to the low center storms. The number of cases in each category is included as an indication of the sampling adequacy.

Unfortunately, the regression curves from which Table 3 was constructed did not produce parallel curves, so that a simple statement of the relative effects of each type cannot be made. Combining all values of P, air mass storms showed the greatest relative variability on all three networks, although static fronts had larger values for very light rains on Little Egypt and there were several cross-overs at the heavier values of P on Panther Creek. The Panther Creek sample was somewhat smaller than those for the other two networks, especially for the heavier rainfalls, so that the cross-overs may be more sampling vagaries than real properties.

Low centers and warm fronts showed the lowest relative variabilities among the storm types in Table 3. These systems are more likely to be associated with widespread precipitation of relatively long duration over a given sampling area than air mass, cold frontal, and squall-line storms which show considerably higher variabilities. Stationary frontal storms, which show high variabilities especially at the heavier rainfall levels, are often of long duration but are frequently associated with intense thunderstorms and rainshowers with steep gradients which tend to produce high relative variability on a given sample area.

Monthly, Seasonal, and Annual Variability

For some purposes, long-term differences in relative variability on a given area over periods of a month, season, or year are of more interest than storm variability. One example is the establishment of sampling requirements for long-term weather modification experiments. Monthly and 3-monthly values of average relative variability on the Boneyard Network of 10 square miles and Little Egypt Network of 550 square miles are shown in Table 4.

The intermonthly and interseasonal differences are considerably greater in the southern Illinois network than in the east-central Illinois network. Also, a pronounced maxi-

TABLE 3.—Synoptic Storm Type Comparisons of Relative Variability.

Storm Type	Number of Cases	Average Relative Variability (%) for Given Mean Rainfall (in.)					
		0.05	0.10	0.25	0.50	1.00	2.00
All Gages							
Little Egypt							
Air Mass	127	97	81	64	54	45	38
Cold Front	235	83	71	49	34	28	21
Warm Front	43	62	52	42	37	30	25
Static Front	182	110	82	54	40	30	22
Low Center—Occluded Front	302	62	45	29	23	15	11
Squall Line	77	130	88	52	34	23	15
Panther Creek							
Air Mass	118	84	57	40	30	23	17
Cold Front	184	56	44	32	25	20	16
Warm Front	49	54	40	27	20	15	11
Static Front	141	64	52	39	31	25	20
Low Center—Occluded Front	184	46	36	27	22	17	14
Squall Line	48	79	57	38	27	20	13
Boneyard							
Air Mass	118	74	53	34	25	18	13
Cold Front	267	41	32	24	19	15	12
Warm Front	60	53	36	21	14	9	6
Static Front	169	55	40	26	19	14	10
Low Center—Occluded Front	362	36	26	18	13	10	8
Squall Line	91	57	38	23	15	10	7

TABLE 4.—Monthly and Seasonal Comparisons of Relative Variability.

Network	Monthly Averages											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Little Egypt.	9	10	9	11	15	18	20	19	23	36	11	8
Boneyard..	9	8	7	7	8	10	10	12	12	8	8	7

Network	3-Month Averages											
	Jan.-Mar.	Feb.-Apr.	Mar.-May	Apr.-June	May-July	June-Aug.	July-Sept.	Aug.-Oct.	Sept.-Nov.	Oct.-Dec.	Nov.-Jan.	Dec.-Feb.
Little Egypt.	10	10	11	15	17	19	20	26	23	18	10	9
Boneyard..	8	7	7	8	9	10	11	10	10	7	8	8

mum from late summer to early fall is indicated in southern Illinois. This maximum reflects a trend for unstable precipitation from showers of small areal extent to dominate the southern Illinois precipitation climate during these months. The Little Egypt Network, as indicated earlier, receives a greater portion of its total precipitation from the highly variable air mass storms than does the Boneyard Network. Effects of these storms, which occur most frequently from spring to fall, are reflected in the greater range of monthly values on Little Egypt.

An examination of months with above and below normal precipitation on the two networks showed the "dry" months with an average V of 19 percent, compared with a "wet" month average of 12 percent on Little Egypt. Similarly, the below normal months averaged 10 percent compared with 7 percent in the above normal months on Boneyard. This reflects the effects of fewer, lighter, and less extensive storms in the dry months. A probability distribution of monthly relative variability, grouped by season, is presented for the two networks in Table 5 to provide a more detailed description of the monthly distribution.

A comparison was made of the annual relative variability on the two networks during the 9-year period, 1958-1966. V averaged 4.4 percent on Little Egypt with a range of 3 to 6 percent, compared with a mean of 3.6 percent and a range of 2 to 6 percent on Boneyard. When 2-year to 5-year moving averages were computed, relative variabilities of 2 to 3 percent were obtained on the two networks. Thus, when precipitation is averaged over several years, the effect of spatial variability becomes minor in regions of approximately homogeneous precipitation climate, and the small values probably reflect sampling errors to a larger extent than true precipitation differences. These findings are useful in several applications, such as the establishment of sampling periods for the determination of precipitation normals and in the planning of precipitation modification experiments.

Conclusions

The spatial relative variability of storm precipitation was found to be related exponentially to mean precipitation. The relation was not significantly improved by adding other variables, such as storm duration and maximum storm precipitation. The relative variability tends

TABLE 5.—Probability Distribution of Monthly Relative Variability Grouped by Season.

	Relative Variability (Percent)							
	Little Egypt				Boneyard			
Probability (Percent)	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
5	18	31	36	109	17	17	27	16
10	16	24	29	53	14	14	21	16
20	13	14	23	31	12	10	14	13
30	10	12	22	28	9	8	11	12
40	9	10	20	24	9	7	10	11
50	9	9	18	16	8	7	10	10
60	8	8	16	12	7	6	8	8
70	7	8	15	10	6	6	7	7
80	6	7	14	9	6	5	6	5
90	5	7	12	6	5	4	6	4
95	4	6	11	6	3	3	6	4

to increase with increasing area and to be substantially greater with unstable types of precipitation (RW, TRW) than with steady types (R, S). Grouped by synoptic weather type, the highest relative variability, in general, was obtained with air mass storms and the lowest with low center passages and warm fronts.

The relative variability of monthly precipitation was found to maximize and minimize in different months of the year between sampling networks, apparently reflecting climatic differences in precipitation from central to southern Illinois. In general, the relative variability was lowest in winter and highest in the warm-season months, and months with below-normal precipitation tended to have higher variability than above-normal months.

Analyses of annual relative variability showed that spatial variability becomes minor in regions of approximately homogeneous precipitation climate when precipitation is averaged over a period of two to five years.

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Manuscript received September 7, 1967.

A COMPARISON OF RAINDROP SIZE SPECTRA BETWEEN MIAMI, FLORIDA, AND CORVALLIS, ORGEON

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ABSTRACT.—Corvallis, Oregon, and Miami, Florida, two locations where raindrop data have been collected using the raindrop camera, a device which photographs raindrops as they fall, have been compared with respect to the raindrop spectra that have been determined with the camera. The spectra were contrasted with respect to total number of drops (N_T) per average rain rate, per cubic meter of sample, geometric mean diameter (D_G), which is associated with a log-normal distribution, mode diameter (D_M), and the diameter of drops at which half the liquid water content lies above that diameter and half below (D_L). Results indicate that, for similar rainfall rates, Corvallis, Oregon has more of the relatively small drops as well as more of the relatively large ones, than Miami, Florida. As a consequence of this, it is found that greater radar reflectivities exist at Corvallis than at Miami for the same or similar rainfall rates.

Much data have been collected with the raindrop camera, as described by Jones and Dean (1953) and Mueller (1960). These data include drop-size distributions for rains in several climatic regions throughout the world, and were obtained in an attempt to establish relationships between radar reflectivity and rainfall rate for these regions.

In the following discussion, a drop-size distribution is characterized by the number of drops in each 0.1 mm size interval from 0.5 mm - 7.9 mm in diameter. To date, all of the analyses have not been completed, although radar-rainfall relationships have been determined for most of the locations. Of course, statements concerning relationships involving rainfall rate (R), and the radar reflectivity factor (Z) must necessarily involve a discussion of the kinds of drop-size distributions that exist during the rains, since Z is directly dependent on D^6, the sixth power of the drop diameters, where:

$$Z = \sum_{D=0.5}^{D=7.9} n_D D^6 \qquad (1)$$

n_D being the number of drops of diameter D. If unique distributions existed in nature for various climatic areas, then unique Z-R relationships would follow. Unfortunately, this is not the case. However, an examination of the raindrop-size spectra that do exist would certainly be of interest and value in any attempt to establish Z-R relationships. Two locations where raindrop data were collected, Miami, Florida, and Corvallis, Oregon, have been compared with respect to their drop-size distributions, and the results are presented herein.

Method of Analysis

Briefly, the raindrop camera takes seven pictures, approximately 1½ seconds apart, at the beginning of a minute, and then becomes inactive for the remainder of the minute. Each frame represents a volume of about 1/7 cubic meter, so one minute of data represents approximately one cubic meter. The drops are measured individually, and their number and size are punched onto data cards.

In order to discover general trends and characteristics associated with the distributions, it was necessary to examine average drop-size spectra in the two locations, rather than individual minutes of data. The averages were determined as follows. The data from each of the two locations were sorted in ascending order according to rainfall rate, and then grouped into intervals 1.0 mm/hr wide at the lowest rates, increasing in size at higher rates. The average number of drops per cubic meter in each 0.1 mm increment of drop diameter from 0.5-7.9 mm, along with other related parameters, was calculated by a computer. For this study, all types of rains were grouped together; there was no stratification of the data according to various rain types or synoptic types, for example, since the purpose of this study was to make general comparisons between the two locations. At Miami, a total of 2506 cubic meter samples were collected, and 1703 cubic meter samples were collected at Oregon. It may be of interest to note that approximately 65 percent of the samples collected at Miami were associated with convective type precipitation, while Oregon samples were on the order of 33 percent convective in nature.

One method of comparing two groups of distributions is to examine the N_T's for the same or similar rainfall rates from the two locations on log-log coordinates, where N_T is the total number of drops per cubic meter of sample for a particular rain rate. Fig. 1 is an example of this where the ordinate represents Oregon, and the abscissa represents Miami. Each point then represents the average total number of drops for each location for the same average rain rate. Now, it is somewhat difficult to point to one parameter and state that it alone, or together with another, describes a particular drop-size spectrum completely, so we should also examine other factors. In Figs. 2A, 2B, and 2C, three comparisons are made between the two areas, on semi-log coordinates, with average rainfall rate as abscissa and D_G, D_M and D_L respectively as ordinate. D_M is the diameter of the mode of the spectrum, which is the

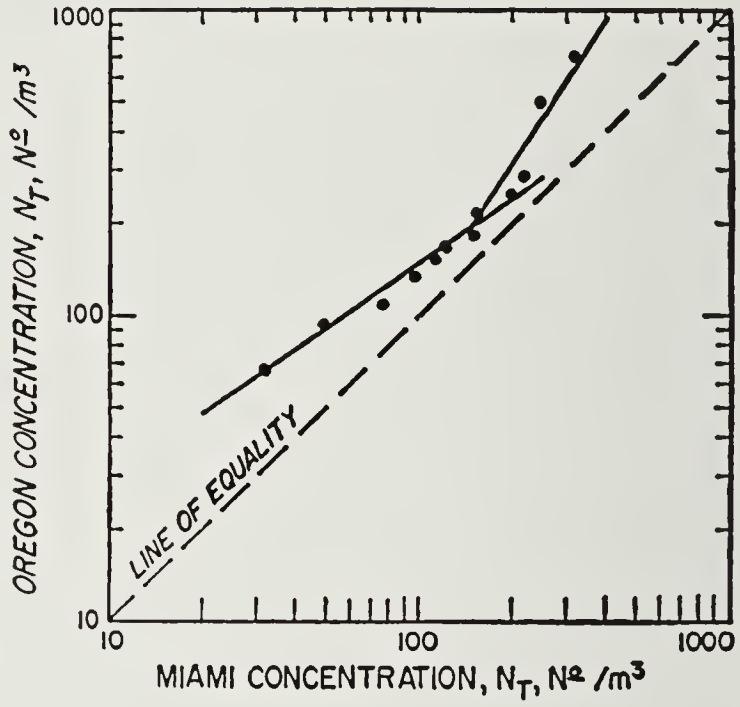


Figure 1. Drop concentrations for Miami and Oregon (from average spectra for all data, no stratification).

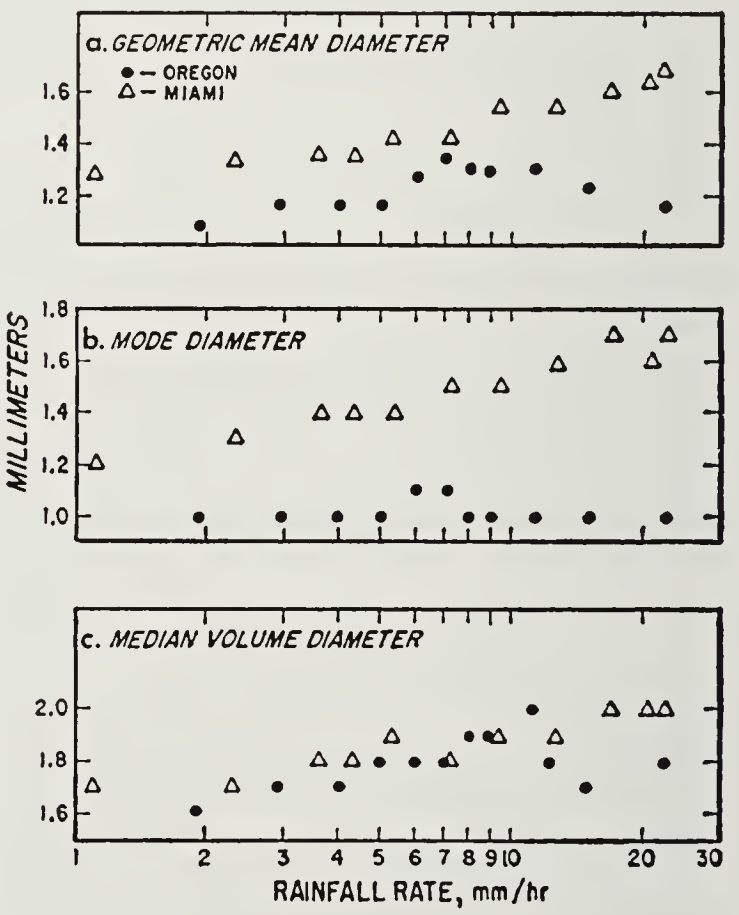


Figure 2. The mode diameter, the geometric mean diameter, and median volume diameter as a function of rainfall rate for Miami and Oregon.

diameter of the size drop that occurs in the largest numbers. D_G is the geometric mean diameter with:

$$\ln D_G = \frac{\sum_{i=1}^{i=n} n_i \ln D_i}{N_T} \qquad (2)$$

as described in an equation for a log normal distribution fit for the drop-size spectra, and D_L is the median volume diameter which is the diameter of the drop-size where half of the liquid water content of the distribution, for the one cubic meter sample, lies above that drop-size and half below. One should also examine the relationship between N_D and D, as in Figs. 3 and 4, where N_D is the number of drops of diameter D. There are other means of comparing spectra, but it is felt that the ones used in this discussion are the most directly related to the distributions.

Results of Analysis

Examination of the N_T comparison between the two locations (Fig. 1) reveals that, for the same rainfall rate, Oregon consistently has more drops than Miami. This would imply that Oregon has smaller drops than Miami, or more specifically, that some average drop-size parameter, such as D_G, D_M or D_L is greater for Miami for the same or similar rainfall rates. In Figs. 2A and 2B this seems to be the case when R is compared with D_G and D_M, respectively; both parameters are consistently higher for Miami for the same or similar rainfall rates. However, D_L for Miami is only slightly higher than for Oregon. (Fig. 2C)

To state flatly that Oregon has smaller drops than Miami would not be describing the situation completely; a more detailed examination is necessary. Figs. 3 and 4 are comparisons of spectra for similar rates from both locations. The particular spectra that were used appear to be representative of their respective areas. These figures indicate that Oregon has more of the smaller size drops, but also more of the larger drops, with a temporary reversal in the vicinity of the Miami mode. This reversal, incidentally, is the reason why the rates are very nearly equal, for, quite obviously, if Oregon had more drops of every size, the rates wouldn't be similar. Therefore, a more accurate statement concerning the two areas would be that Oregon has both more of the relatively small and relatively large drops, rather than that Oregon has smaller drops.

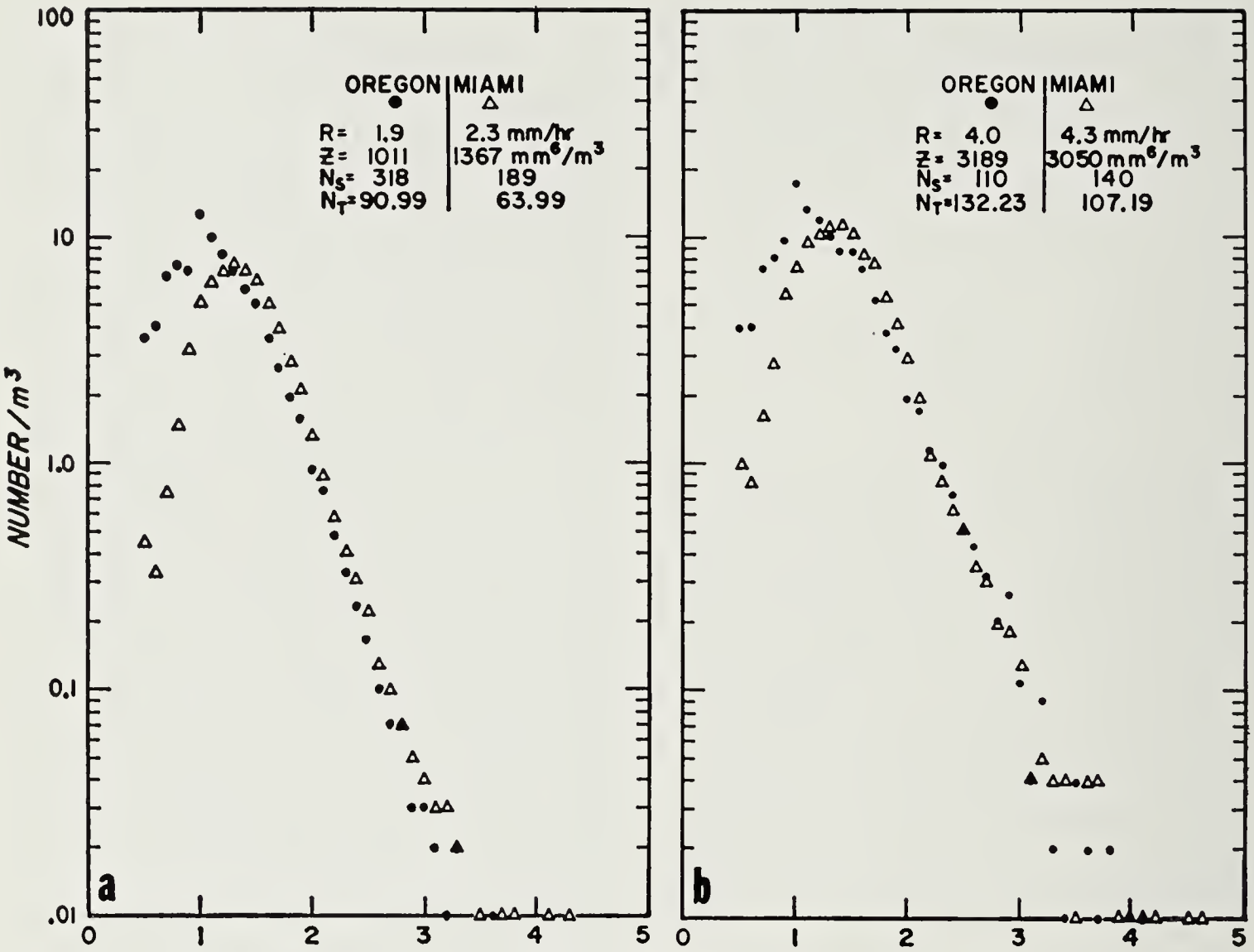
In addition, an examination of the Z-R relationship from both locations reveals that for equal rainfall rates, Oregon has greater reflectivities for R greater than 0.8 mm/hr. The actual Z-R equations for both regions are as follows; for Oregon,

$$Z = 301\ R^{1.64} \qquad (3)$$

for Miami,

$$Z = 286\ R^{1.43} \qquad (4)$$

where Z is in units of mm^6/m^3 and R is in mm/hr. On a plot of R vs. Z on log-log coordinates, the linear relationship for Oregon has a greater slope and a greater Z-intercept (at R = 1.0 mm/hr) than Miami, as is revealed in Fig. 5. This means that for equal rainfall rates the reflectivities for Oregon are indeed greater. It may be concluded from this, that Oregon has larger drops than Miami, which agrees with the above analyses, although it was



DROP DIAMETER, mm

FIGURE 3. Raindrop spectra from Miami and Oregon.

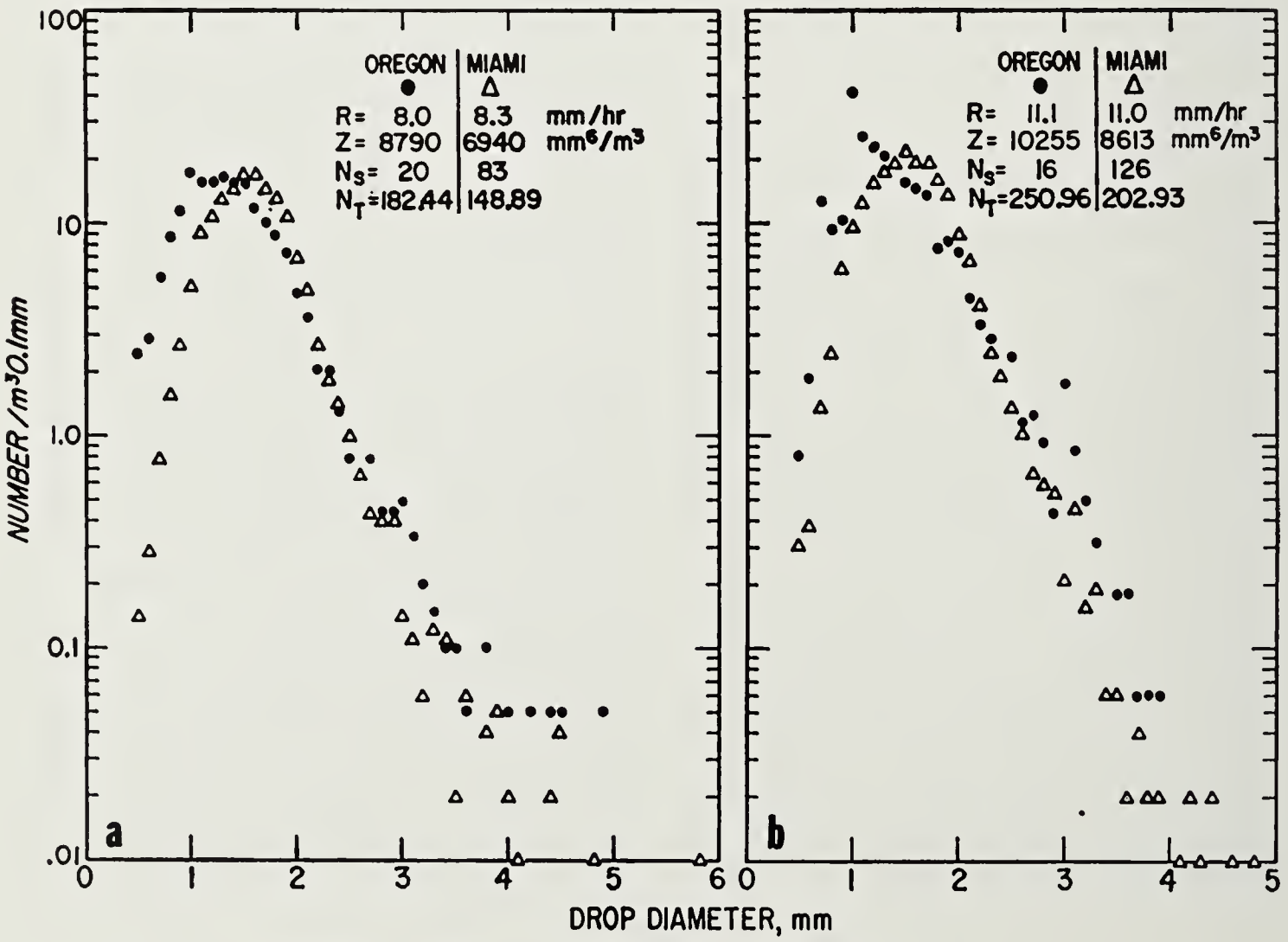


FIGURE 4. Raindrop spectra from Miami and Oregon.

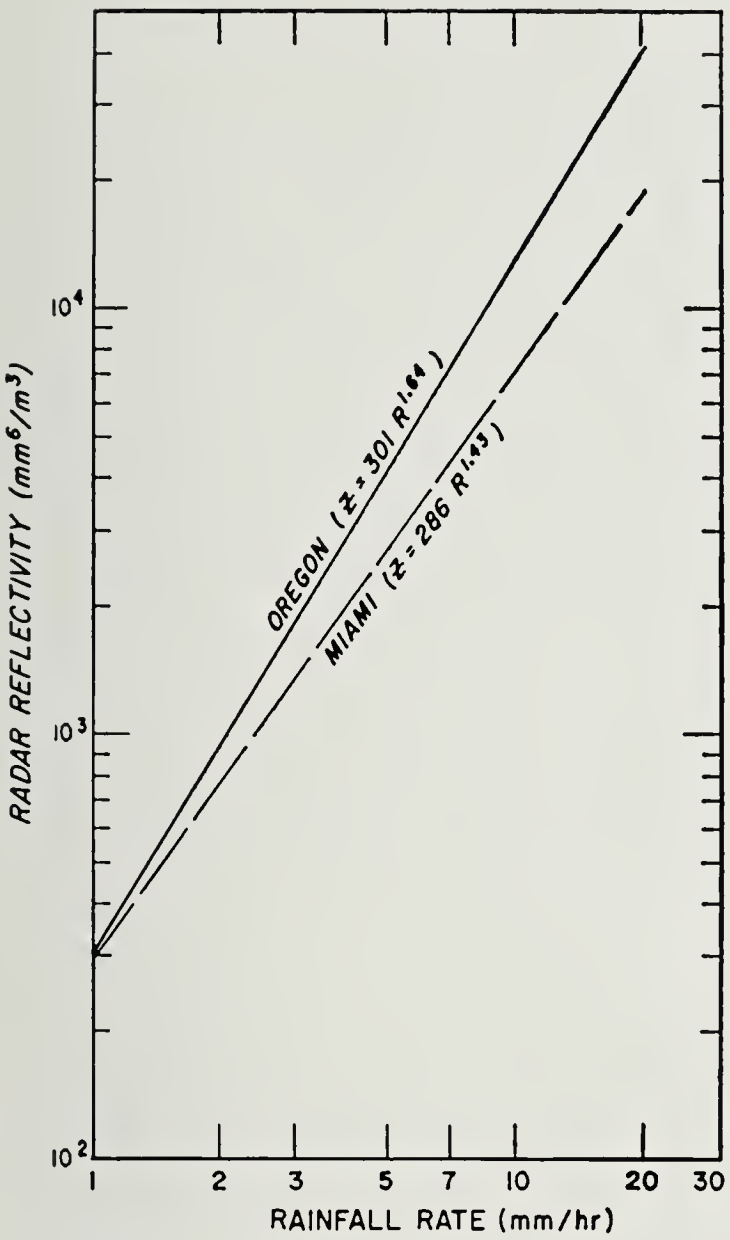


FIGURE 5. Z-R regression lines for Miami, Florida and Corvallis, Oregon.

found upon examining the actual drop distributions that in addition, Oregon has more of the smaller drops. So apparently, the greater number of larger drops in Oregon represents a sufficient enough difference to result in greater reflectivities, since Z depends directly on D^6, but not large enough to produce greater D_G, D_M and D_L values which are linearly dependent upon D. This agrees with the fact that the N_T's at Oregon are greater than at Miami for the same or similar rates. The standard error of estimate for the Miami regression line in Fig. 5 is 0.198 and for Oregon it is 0.136. A standard error of estimate of 0.136 on R means that for a given measurement of Z an error of less than $\pm$ 37 percent on R can be expected 68 percent of the time.

It should be pointed out that the number of samples (N_S) used from both areas in Figs. 3 and 4 varied substantially, with Miami having four times the number of Oregon in Fig. 4A, and eight times the number in Fig. 4B. There are two reasons for this. First, more data were collected at Miami, and secondly, much of the data taken at Oregon were rains of very low rates; for example, approximately 70 percent were rates of less than 2 mm/hr. It is believed, however, that the number of samples taken at Oregon is adequate for a valid comparison. It should also be noted that many of the corresponding N_T's for the relationship in Fig. 1 were arrived at by interpolating between rates on a R-N_T curve in order to obtain the same rainfall rate for both locations when attempting to establish a point on the N_T-N_T curve.

SUMMARY

The drop-size spectra at Oregon and Miami were compared with respect to D_G, D_M, D_L and N_T. The investigation revealed that Oregon rains have larger numbers of drops for the same rainfall rates. Upon closer examination, it was found that the Oregon rains have more of the smaller drops as well as more of the larger ones. The parameters used for the comparison are not necessarily the only ones that are available for valid analyses, but to date appear to be the most appropriate. A similar type of study is under-

way for the other locations where raindrop-size distribution data have been collected, and the results from those will be available in the near future.

Acknowledgments

This study was done under the supervision of Mr. G. E. Stout and Dr. E. A. Mueller. I would like to thank Mr. Stout, Dr. Mueller, and Mr. A. L. Sims for their valuable suggestions and discussions.

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Manuscript received July 1, 1967.

SPECIES DIVERSITY OF BENTHIC MACROINVERTEBRATES IN THREE MOUNTAIN STREAMS

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ABSTRACT.—Species diversity of benthic macroinvertebrates in three unpolluted mountain streams was determined by using information theory methods. Results were compared with previous studies of a lowland, non-polluted stream, and two lowland, polluted streams. Maximum diversity was greater in mountain streams than in a grossly polluted stream but compared favorably with the furthest downstream station in a stream receiving oil field brines.

Indices derived from the field of information theory have been utilized in the analysis of benthic macroinvertebrate community structure by a number of investigators. Wilhm and Dorris (1966) utilized this approach in their study of a stream receiving sewage and industrial wastes. Mathis and Dorris (1968) utilized the methods in a study of an oil field brine polluted stream while Harrel and Dorris (1968) utilized information theory methods in a study of an unpolluted stream within one drainage basin. In each case, estimates of community structure were obtained with Patten's (1962) equations:

$$(1)\quad \overline{H} = \sum_{i=1}^{m} \frac{ni}{\overline{N}} \log_2 \frac{ni}{\overline{N}}$$

(Diversity per individual)

$$(2)\quad H\ max = \log_2 N! - m\ \log_2 \times (N/m)!$$

(Maximum diversity)

$$(3)\quad Hmin = \log_2 N! - \log_2 \times [N-(m-1)]!$$

(Minimum diversity)

$$(4)\quad R = \frac{Hmax - H}{Hmax - Hmin}$$

(Redundancy)

where (N) is the total number of organisms, (ni) number of individuals per species and (m) number of species in a unit area. Calculations were performed on an IBM Type 1620 data processing machine at the Bradley University Computing Center.

In using these equations, diversity and information are considered to be synonymous and calculations are made directly from numbers of individuals. Diversity, or information, is expressed in bits (short for binary digit), one bit being the amount of information required to specify one of two equally probable states. Information theory indices do not attempt an explanation of observed phenomena. Rather, they estimate the amount of information required to define community structure (Hairston, 1959).

Polluted streams should have less diversity than non-polluted streams

since some species are unable to survive. The remaining species encounter less competition and are able to produce large numbers of individuals if sufficient nutrients are available. The decreased numbers of species and increased numbers of individuals in the polluted community represent fewer bits of information.

A natural community, on the other hand, tends toward a more complex assemblage. Because spring fed mountain streams in remote and sparsley populated areas are relatively free of harmful effluents, they provide extremely stable habitats. Thus, benthic macroinvertebrate communities found in streams of this type reflect an increased ecological stability in terms of high $\overline{H}$ and low R.

Although streams exhibit an almost infinite variability, certain habitats such as riffles, runs, and pools are characteristically present. Species extant in these habitats are different throughout the world, but the main taxa, or their ecological equivalents, are similar (Harrel and Dorris, 1968). With the use of information theory indices, an investigator is able to make objective comparisons of community structure between different streams in different parts of the world.

In the present study, species diversity of benthic macroinvertebrates was determined during the summer of 1966 for three unpolluted mountain streams in southwest Virginia. The results were compared with previous studies of a lowland, non-polluted stream and two lowland, polluted streams in Oklahoma, Otter Creek, and Black Bear Creek and Skeleton Creek, respectively. Black Bear Creek was polluted with oil field brines; Skeleton Creek was polluted with domestic and oil refinery effluents.

Description of Study Area

Three small mountain streams, White Rock Branch, Spruce Run, and Little Stony Creek located in Giles County, Virginia were selected for study. They were typical mountain streams with long riffles and short pools. All three streams were spring-fed and maintained an almost constant flow during the study. Stream width ranged from 3.6 m to 1.8 m (Table 1). Sampling sites ranged from an altitude of 533 m to 834 m.

Physico-chemical conditions were measured only once on 2 August 1966

Table 1.—Station Characteristics.

Stream	Station	Width (m)	Depth (m)	Velocity (m/sec)	Discharge (m^3/sec)
White Rock Branch	Upper	1.8	.09	.51	.06
	Lower	2.7	.08	.51	.12
Spruce Run	Upper	1.8	.08	.38	.08
	Lower	2.4	.15	.32	.10
Little Stony Creek	Upper	3.6	.13	.29	.11
	Lower	3.6	.20	.29	.17

(Table 2). The low pH in Little Stony Creek can be attributed to the presence of a bog located near the upper reach of the stream. The low pH reading is also reflected by the low alkalinity. Dissolved oxygen was near 100% saturation at all stations and ranged from 93% saturation to 105% saturation.

Procedures

Ten bottom samples were taken with a Surber sampler from each station between 28 July 1966 and 10 August 1966. Five riffle samples were collected approximately one-half meter from the shore and five were taken from the middle of the stream. Pool samples were not taken. Rocks and gravel were removed from the sample area, placed in a large bucket and individually brushed with a paint brush. Samples were then washed in a sieve (U.S. standard soil series with openings of 0.420mm) and preserved in 70% isopropyl alcohol. Crayfish were not collected. Alkalinity and dissolved oxygen were measured in the field according to standard methods. The pH was measured by means of a portable Sargent pH meter. Stream discharge was estimated by the method of Robins and Crawford (1954).

Discussion

The majority of species collected during the study were dipterans, caddisflies and mayflies (Table 3). These taxa dominated the fauna at all stations. Most of the dipterans collected during the study were tendipedid dipterans. At most stations, tendipedids provided a disproportionate share of the species but few individuals per species.

Taxa represented by the greatest numbers of individuals included the caddisflies, mayflies and stoneflies (Table 4). Other taxa present in abundance included elmid larvae and tendipedid dipterans. The elmid larvae reached maximum density at the lower Station on Spruce Run where they comprised approximately 25% of the total numbers of individuals. Psephenid larvae were abundant only in Spruce Run. This stream exhibited the highest water temperature as well as the highest alkalinity and was located at a slightly lower altitude than the other two streams.

Spruce Run was the most productive mountain stream in terms of individuals per m^2 (Table 5). By comparison, Black Bear Creek was even more productive since large numbers of *Cheumatopsyche* sp. were present during the summer (Mathis

Table 2.—Physico-Chemical Conditions in Three Mountain Streams.

Stream	Station	Temp. C	pH	Alkalinity		Oxygen ppm
				HCO_3^- ppm	$CO_3^=$ ppm	
White Rock Branch..	Upper.....	16	8.3	52	0	9.9
	Lower.....	16	7.8	58	0	11.0
Spruce Run.....	Upper.....	21	7.5	115	0	8.4
	Lower.....	19	8.4	128	0	9.9
Little Stony Creek...	Upper.....	16	5.0	12	0	9.3
	Lower.....	16	6.7	13	0	10.0

TABLE 3.—Species Distribution in Three Mountain Streams.

Stream	White Rock Branch		Spruce Run		Little Stony Creek	
Station	Upper	Lower	Upper	Lower	Upper	Lower
Diptera	13	29	24	23	14	22
Trichoptera	10	15	11	11	9	6
Ephemeroptera	10	17	14	13	5	9
Plecoptera	7	3	6	7	3	5
Coleoptera	3	4	6	6	3	5
Annelida		1	1	3	1	4
Pelecypoda		1	1		1	
Megaloptera		1	2	1	2	1
Odonata	1		2		1	
Gastropoda			3	2	1	
Turbellaria			1	1		
Crustacea			1			
Total Species	44	75	69	63	39	54

TABLE 4.—Relative Abundance of Dominant Benthic Macroinvertebrates in Three Mountain Streams.*

Stream	White Rock Branch		Spruce Run		Little Stony Creek	
Station	Upper	Lower	Upper	Lower	Upper	Lower
Taxa						
Trichoptera	214	945	746	1,040	301	208
Ephemeroptera	357	443	863	806	44	175
Plecoptera	493	305	210	128	281	75
Elmidae	34	72	684	1,179	513	8
Psephenidae			484	903		
Tendipedidae	11	76	232	56	409	79

* Per m^2

TABLE 5.—Species Diversity Range in Polluted and Unpolluted Streams During Summer.

Source	Sampling Sites	Individuals/M^2	Number of Species	$\overline{H}$ Bits/Ind.	R
Unpolluted					
White Rock Branch, Va., (Present Study)	Riffles	1,149–1,990	44–75	4.14–4.33	.25–.30
Spruce Run, Va., (Present Study)	Riffles	3,571–4,248	63–69	3.60–4.12	.33–.41
Little Stony Creek, Va., (Present Study)	Riffles	679–1,660	39–54	3.71–4.61	.17–.29
Otter Creek, Okla., (R. Harrel, personal communication)	Pools and Riffles	280–2,633	23–43	2.66–3.53	.32–.58
Polluted					
Black Bear Creek, Okla., (Mathis and Dorris, 1968)	Pools and Riffles	213–6,748	13–41	2.30–3.93	.20–.58
Skeleton Creek, Okla., (Wilhm and Dorris, 1966)	Pools and Riffles	150–3,022	5–16	0.55–3.09	.19–.89

and Dorris, 1968). While a few taxa dominated the community to some extent in the mountain streams, other species were not excluded as is the case in polluted streams. As a result, maximal values for $\overline{H}$ were greater in mountain streams than in the polluted lowland streams. Maximal $\overline{H}$ in Black Bear Creek, however, exceeded minimal $\overline{H}$ in two of the mountain streams. The high $\overline{H}$ in Black Bear Creek was found at the extreme downstream station where brine concentration was lowest.

The range in values for $\overline{H}$ in Otter Creek, the only unpolluted lowland stream that has been subjected to information theory methods to date, closely paralleled those of Black Bear Creek. The range reported for Skeleton Creek, on the other hand, was considerably greater. This is an indication that oil field brines in Black Bear Creek were less restrictive to populations of bottom organisms than domestic and oil refinery effluents in Skeleton Creek (Mathis and Dorris, 1968).

R, a measure of faunal repetition, was minimal at the lower Station in Little Stony Creek but it was also the least productive in terms of individuals per m^2. The bottom fauna extant in this stream was more equitably distributed among the species, thus R was low and $\overline{H}$ was high.

Highest R, on the other hand, was found in Spruce Run where large numbers of riffle beetles, caddisflies and mayflies were found.

Minimal R in the polluted streams was comparable to minimal R in all other streams. The low values for R in the polluted streams were obtained at extreme downstream stations where clean water conditions were present. Maximal R in polluted lowland streams exceeded maximal R in all three mountain streams. R in both Black Bear Creek and Otter Creek was 0.58. The similarity of values for this parameter lends support to the contention that faunal compositions in the two streams are not extremely dissimilar, despite the presence of oil field brines in Black Bear Creek.

Maximum values for $\overline{H}$ exceeded 4.00 in all three mountain streams. On the basis of this investigation, it would appear that maximum $\overline{H}$ for benthic macroinvertebrates in habitats of this type lies somewhere between 4.00 and 5.00, at least during summer. Comparative data from other seasons is lacking.

Acknowledgments

This study was supported by an NSF post-doctoral research grant GB-3439 administered by the University of Virginia.

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Manuscript received October 7, 1967.

THE USE OF POLYACRYLAMIDE GEL ELECTROPHORESIS IN THE IDENTIFICATION OF *XANTHOMONAS FRAGARIAE KENNEDY AND KING*

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ABSTRACT.—Electrophoretic patterns of buffer-soluble proteins extracted from bacterial cells were used as an aid in the precise identification of organisms responsible for the angular leaf spot disease of strawberries. Isolate K-1 was found identical to *X. fragariae* and both of them entirely differed from isolate K-2 in respect of number, position, width, and density of the bands. The usefulness of the technique as an adjunct to other criteria used in the diagnostic investigations and taxonomic studies is stated.

A bacterial disease of strawberry with symptoms suggestive of those caused by *Xanthomonas fragariae* were observed in epiphytotic proportions on variety Dixieland in Kentucky during June, 1965 (Powell and Khare, 1967). Isolations from infected leaves appeared to be easy to obtain but typical symptoms were not obtained from greenhouse inoculations and biochemical tests of the isolate did not conform to those described by Kennedy and King (1962). Further studies showed that a contaminant was involved in the isolation. Thus, two different bacteria, Kentucky-1 (K-1) and Kentucky-2 (K-2) were separated by dilution and studied. K-1 was very similar to *X. fragariae* in physiochemical properties, and when used in pure culture produced fairly typical symptoms of the disease when plants were inoculated. Isolate K-2 differed from K-1 in both cultural and biochemical properties and also proved innocuous to strawberry (Powell and Khare, 1967).

Currently considerable attention is being paid to the use of gel electrophoresis in clinical diagnosis and in numerous other biochemical diagnostic investigations in different fields (Gottlieb and Hepden, 1966; Kates and Goldstein, 1964; Levitron, Myers and Grunbaum, 1964; Macko, Novacky and Stahmann, 1967; Vaughan, Waite, Boulter and Waiters, 1965). Polyacrylamide gel electrophoresis was implemented as an additional tool for the precise identification of bacteria. Electrophoretic patterns of the soluble proteins of K-1 and K-2 were compared with the protein patterns of a pure culture of *X. fragariae* (Minnesota isolate) which was obtained from B. W. Kennedy, University of Minnesota.

MATERIALS AND METHODS

The organisms were purified and cultured on Modified Emerson's medium (MEM) of the following composition (Reinhardt and Powell, 1960):

Yeast extract, 1.0g; peptone, 4.0g; beef extract, 4.0g; dextrose, 1.0g; NaCl, 2.5g KH_2PO_4, 5.0g; K_2HPO_4, 8.0g; agar, 20.0g; distilled water to one liter.

A loop of bacteria from 48 hours-old MEM plates was used to inoculate 50 ml of MEM broth (MEM minus agar), con-

tained in 125 ml Erlenmyer flask. After 12 hours of incubation the inoculum was added to another 2 liter Fernberg flask containing 900 ml MEM broth which was then agitated over a reciprocating shaker (90 cycles/min.) for 48 hours at room temperature (25° - 26° C).

Bacteria were harvested by centrifugation at 3500 x G for 20 minutes. The supernatant was discarded. The pellet was washed three times in phosphate buffer (0.1M; pH 7.0) to remove the nutrients. Bacteria were suspended in the chilled buffer, 4 ml, and subjected to sonification (rupturing the cell wall by sonic oscillation) at 0°C for 10 minutes. The extracts were centrifuged at 105,000 x G (Spinco Ultracentrifuge Model L, Rotor No. 40) for one hour at 4° C. The resultant clear supernatant was decanted and the volume of each sample required to give about 125 ug protein for electrophoresis was determined by Lowry's Folin test (Lowry, Rosebrough, Farr and Randall, 1951).

The disc electrophoresis apparatus (Model 12), materials sold as a 600-pack unit No. 400 P, by CANALCO Corporation, Bethesda, Maryland, was used. The procedure was used as described by Davis (1964) with some modifications (Gill and Powell, 1967).

Six replications were observed in each run of each isolate, and several runs were made from each protein preparation. The experiments were repeated with fresh, and frozen extracts prepared at entirely different times.

Results and Discussion

Investigations were first conducted on the reproducibility of the fractionation method. Electrophoretic protein patterns of each isolate examined were found identical when tested under standardized conditions regardless of whether the extract was fresh or frozen. The reproducibility of the patterns was further checked and found consistent when an extract preparation of a different culture of the same isolate but grown at different times was employed. This substantiates the earlier studies (Chang, SRB and Steward, 1962; Steward and Barber, 1964) that showed reproducibility of the protein patterns of *Neurospora crassa*.

Data (Fig. 1 and 2), based on number, position, density, and width of the band, demonstrated that isolate K-1 was similar to Minnesota isolate (*X. fragariae*) in its electrophoretic pattern. Also, both of them differ entirely from isolate K-2 in this respect. Some similarities in the protein patterns, band P, Q, and

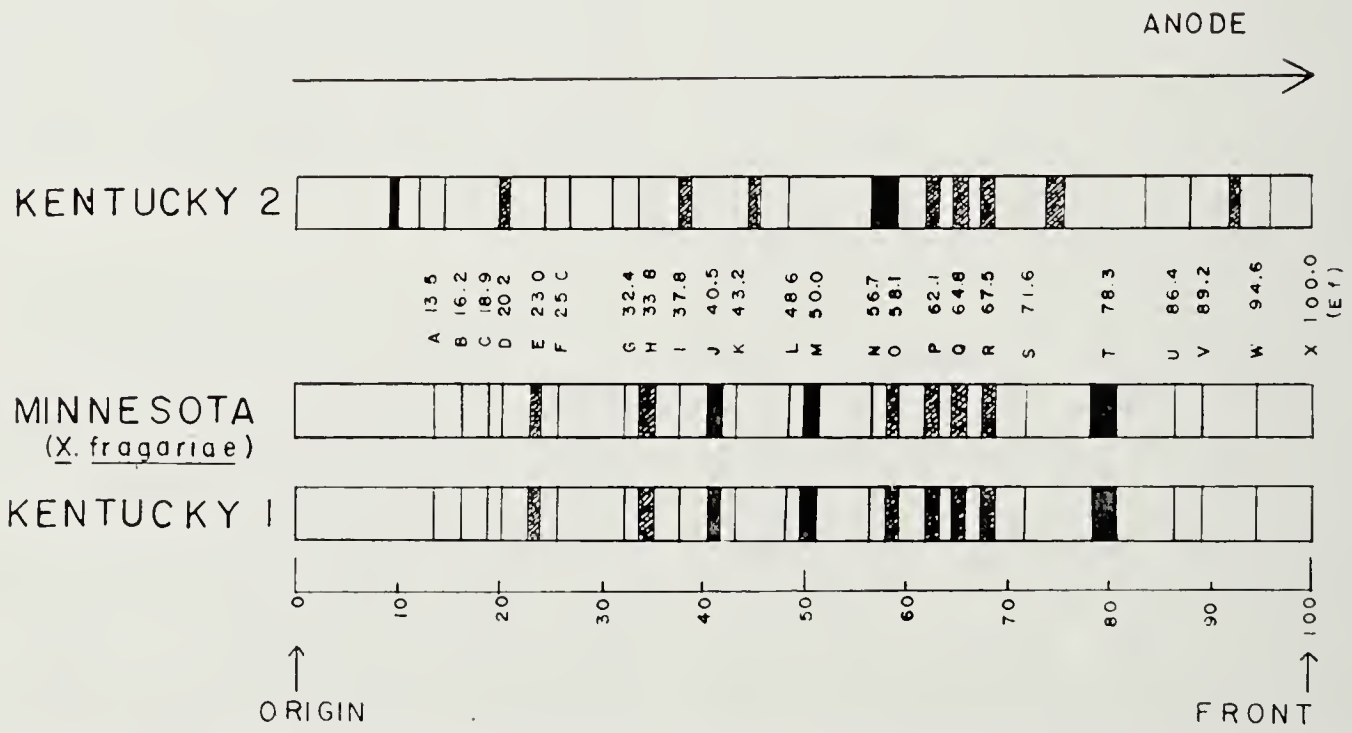

Figure 1. Diagramatic presentation of the electrophoretic patterns of buffer-soluble protein extracts of isolates K-1 and K-2 compared with a Minnesota isolate (*X. fragariae*).

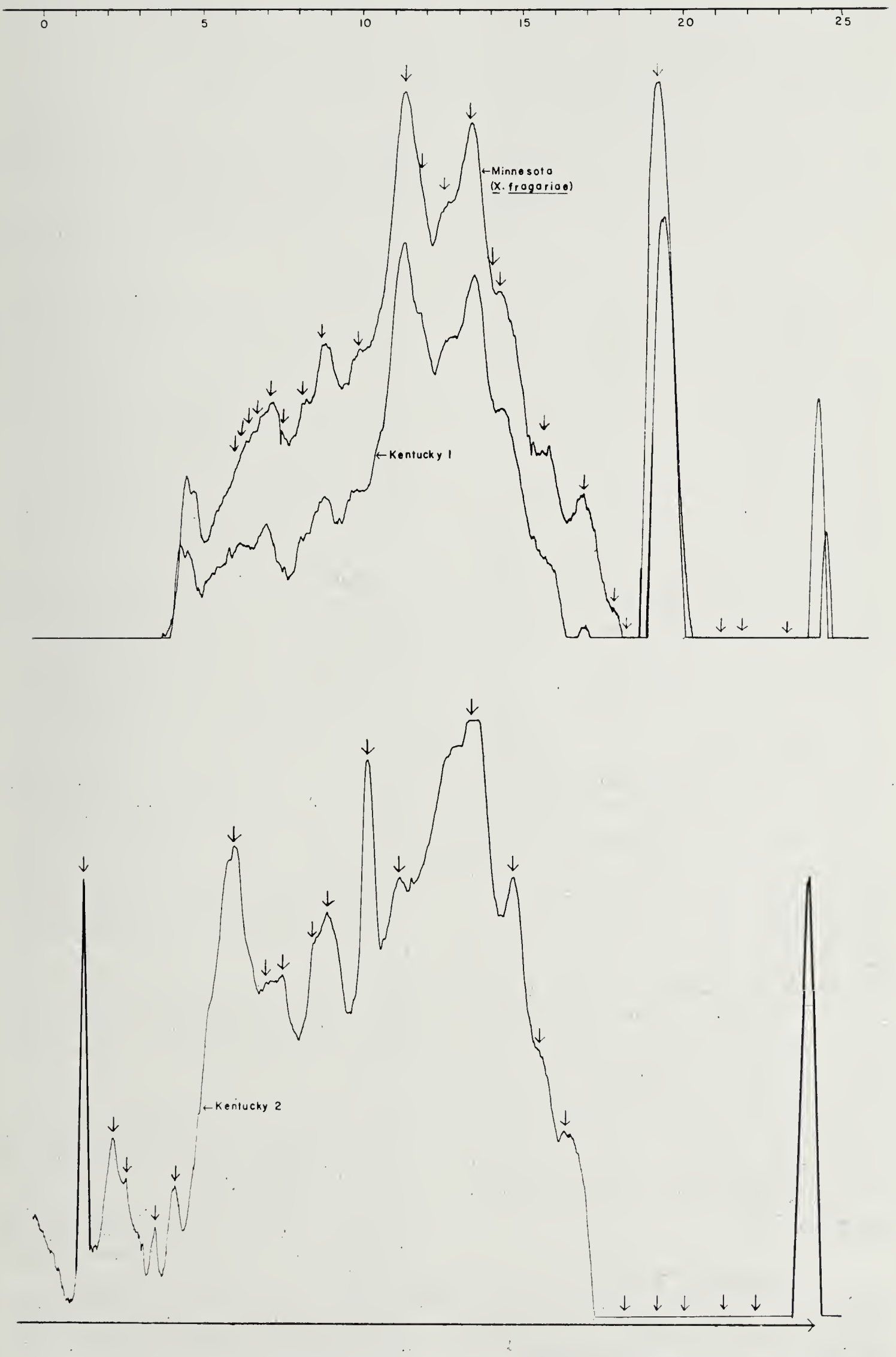

FIGURE 2. Top: A composite of microdensitometric scans of electrophoretic

separation of the buffer-soluble proteins of isolate K-1 compared with Minnesota isolate (*X. fragariae*).

Below: A microdensitometric scan of the buffer-soluble proteins of K-2 isolate bearing little similarities with the other two isolates.

R, appeared identical within the three isolates. However, this does not warrant considering K-2 as another isolate of *X. fragariae*. Identical or nearly identical protein patterns of several isolates within the species have been reported in *Agrobacterium* (Huisingh and Durbin, 1965), *Erwinia amylovora* (Smith, 1967) and in fungi (Clare and Zentmyer, 1966; Durbin, 1966; Gill and Powell, 1967). In contrast to the above results Gottlieb and Hepden (1966) observed variations in the electrophoretic behavior of isolates of a single species of *Streptomyces*. It is strongly emphasized that only organisms in pure culture should be tested by this technique.

The observations presented in this paper support the fact that K-1 is another isolate of *X. fragariae* whereas K-2 is a different bacterium and is a contaminant bearing no resemblence to *X. fragariae* physiologically, pathologically, (Powell and Khare, 1967) or electrophoretically.

The consistent reproducibility of this technique, the identical behavior of the isolates within a species, *X. fragariae*, and the differences detected between species suggest that polyacrylamide gel electrophoresis is a useful technique for the precise identification of phytopathogenic bacteria and it should prove a useful adjunct in taxonomic investigations.

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Manuscript received October 10, 1967.

PETROGRAPHY OF SELECTED CHESTERIAN CARBONATES (VISÉAN-NAMURIAN) FROM THE TYPE AREA IN SOUTHWESTERN ILLINOIS

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ABSTRACT.—Six carbonate sections of the upper half of the Chester Series (Glen Dean Formation to Kinkaid Formation) in type area of Southwestern Illinois were sampled at an average interval of 15 centimeters. Nearly four hundred thin sections were analyzed in a statistical petrographic (microfacies) investigation. Frequency indices for crinoid and bryozoan debris, ostracods, calcified monaxonic sponge spicules, arenaceous foraminifers, calcispheres, detrital quartz and oolites were measured. Indices of clasticity were also computed for crinoid debris, detrital quartz and oolites. Curves of the stratigraphic variation of each parameter were plotted and used for the interpretation of the environment of deposition.

The statistical data combined with textural properties and degree of recrystallization revealed an association, at times regularly cyclical, of five distinct and environmentally controlled microfacies. These are in order of increasing energy level or decreasing relative depth: calcisiltite with scattered debris, mud-supported biocalcarenite and three types of grain-supported biocalcarenites with calcisiltite matrix recrystallized to a variable degree.

All these microfacies indicate an environment of general low energy in which moderate winnowing processes were active. In the shallower portions where crinoid and bryozoan colonies thrived, they led to the inflow of transported oolites, the sorting of crinoid debris and the fragmentation of bryozoan fronds almost in place. In the deeper portions of the environment where abundant siliceous sponges and scavengers were associated with small crinoid clumps, the winnowing processes concentrated the silt-size detrital quartz and all the small organic components such as arenaceous foraminifers, ostracods and calcispheres originally distributed throughout the environment.

A detailed petrographic and environmental study of the limestone units of the Upper Chester series was undertaken in the type area of southwestern Illinois. The units chosen for this study ranged stratigraphically upward from the Glen Dean Formation to the Kinkaid Formation. The published sections from a previous papers by Rexroad (1957) on the conodont fauna of the Chester were used as stratigraphic reference.

Nearly four hundred vertically oriented specimens were collected from six sections at a sampling interval of approximately 15 centimeters. Thin sections were then prepared, investigated petrographically, and divided into six distinct microfacies. Variation curves for the organic and mineral parameters measured in thin section were drawn parallel to the stratigraphic column. From these curves the relative energy level and bathymetry of the different microfacies were interpreted and the detailed vertical evolution of the carbonate sediments in Late Chesterian time was reconstructed.

LOCATION

This investigation was conducted in the vicinity of Chester, Illinois, approximately 60 miles southeast of

St. Louis on the east bank of the Mississippi River. The six sections collected were located in active or abandoned quarries, or in railroad or road cuts (FIGURE 1).

Stratigraphy and Previous Work

D. H. Swann (1963) established a detailed stratigraphy of the Chester series (FIGURE 2) and reviewed all previous stratigraphic work and related terminology. Therefore, it is sufficient to point out here that he has only defined additional members in the type area (pp. 10, 40-42).

Although many workers have contributed substantially to the stratigraphy and paleontology of the Chester in the type area, no detailed petrographic work has been done. Rexroad (1957) described eighteen sections in the type area for an investigation of the conodont fauna. Five of his sampling localities from the Upper Chester were chosen for the present study on the basis of their containing vertically continuous limestone units. They include parts of or all of the Glen Dean, Vienna, Waltersburg, Menard, Clore, and Kinkaid Formations. For comparative purposes Rexroad's original sampling locality numbers were retained in the present study (FIGURE 2), however, locality 10A, Coles Mill South, was chosen as an alternate since the original section has been covered by road construction.

Methods and Techniques

The low regional dip to the east of the Mississippian rocks in the vicinity of Chester greatly facilitated the measuring of the selected exposures. The normal procedure was to take a vertically oriented limestone specimen every fifteen centimeters. Wherever bedding was quite massive, this interval was extended up to thirty centimeters; wherever the layers were eight centimeters or less the entire bed was collected. Sandstones and shales were

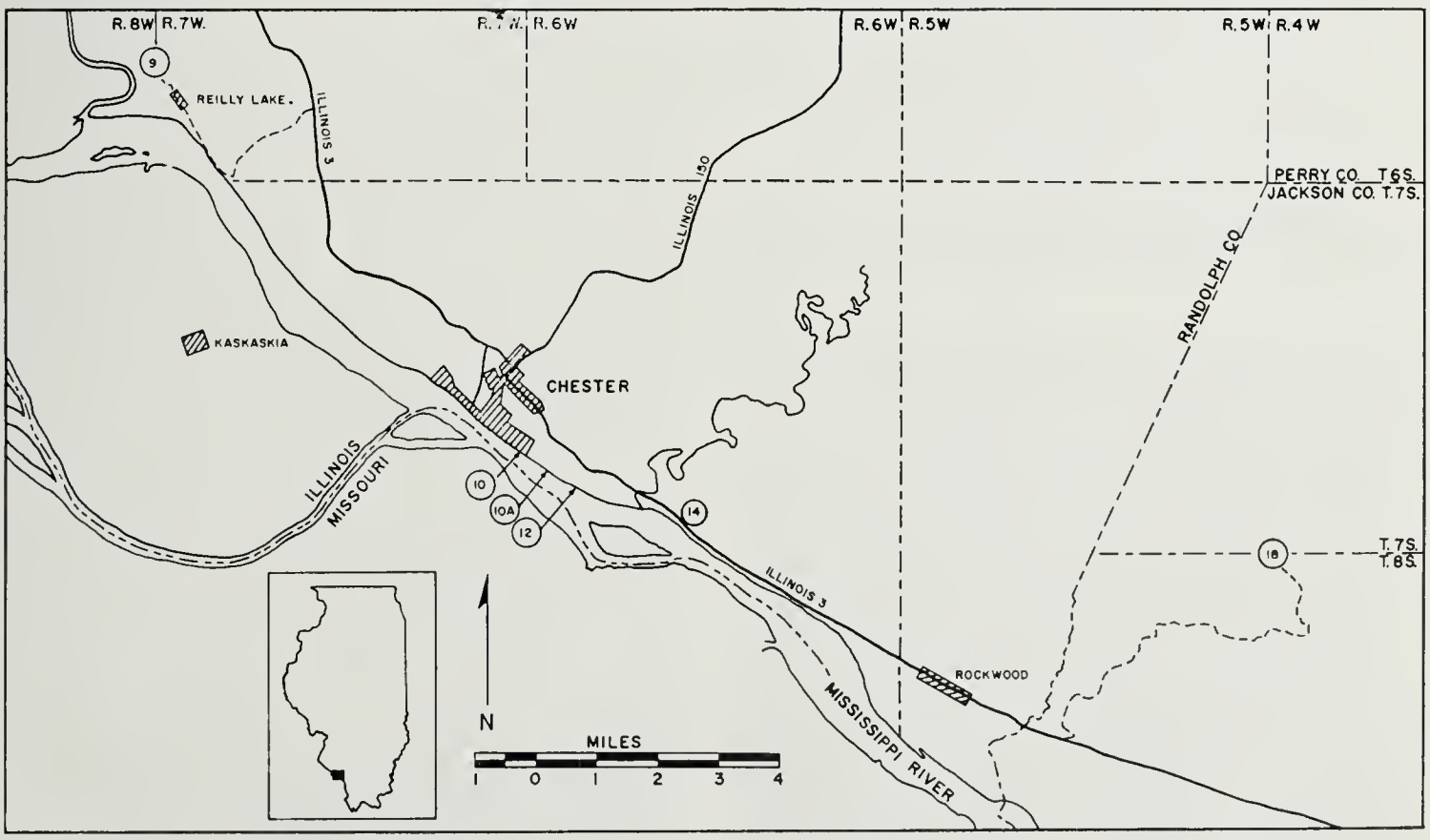
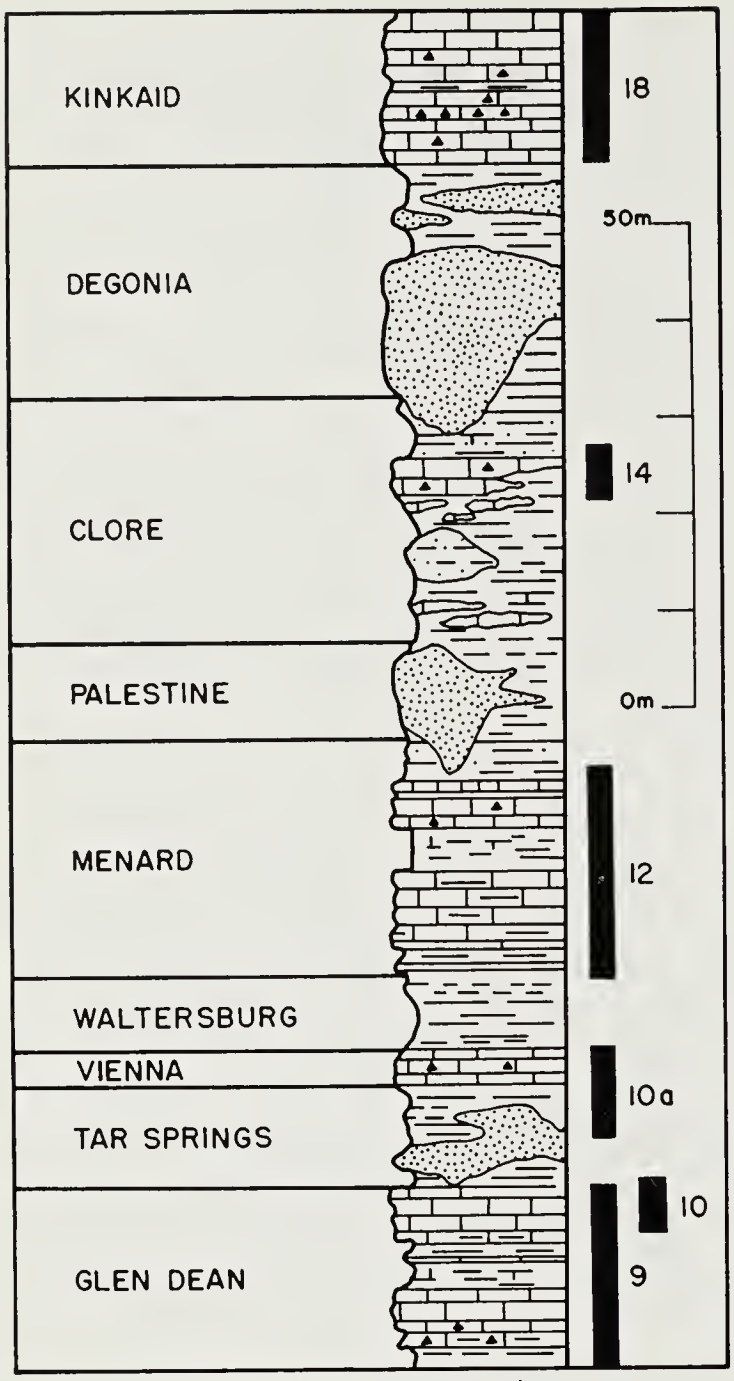


FIGURE 1. — Location map



(MODIFIED AFTER REXROAD, 1957)

GENERALIZED GEOLOGIC COLUMN
OF THE UPPER CHESTER SERIES
IN THE TYPE AREA

FIGURE 2.—Generalized geologic column of the Upper Chester Series in the type area.

also routinely sampled, although at an interval of one to two meters, but were not petrographically investigated.

Approximately 380 specimens of carbonates were collected at an average interval of eighteen centimeters. The slabbed and trimmed specimens were placed in a General Electric XRD-4 diffractometer and scanned from 22 to 40 degrees 2θ. This range includes the major peaks of quartz, calcite, and dolomite. This analysis has shown that nearly all specimens consist of relatively pure limestone with insignificant amounts of dolomite. The thin sections were prepared in the routine fashion except for the use of an Ingram-Ward thin section machine.

They were then studied following the method proposed by Carozzi (1950, 1958, 1961) which consists of measuring the indices of clasticity and frequency of all the detrital components and the frequency indices of all the organic components of a carbonate rock.

The index of clasticity for any given component in a thin section is defined as its maximum diameter. Since we are dealing with an apparent diameter, except in the particular case where the section is cut through the center of the grain, the index of clasticity is the lowest numerical value of the maximum diameter. In order to attain some degree of constancy in the measuring of the index of clasticity, the ten largest grains from all the grains occurring in a particular thin section were selected and their diameters averaged. The occasional isolated grains noticeably larger than the grains making up the rock were considered to be due to local variations in the agents of deposition, and hence, though their diameters were measured, they were not entered into the tabulation of the index of clasticity.

The final expression of the index of clasticity is a numerical figure, given in millimeters, which represents the largest diameter of a grain that could be set in motion by the general forces acting in the environment of deposition.

The frequency of a given component is defined as the number of its particles present in any given area of a thin section. In practice, this requires counting the number of grains found over a constant area on each thin section. This constant area must be maintained in order to achieve the reproducibility of results necessary for the acquisition of statistical validity. A problem arises with the determination of the frequency index. If the rock was made up predominantly of closely packed grains of a particular clastic component, as the grain size increases the frequency index will decrease, thereby giving a misleading value. In order to overcome this, it was decided to choose an area for each clastic component which had a diameter of at least 10 times the average diameter of that component. For crinoids, bryozoans, ostracods, sponge spicules, arenaceous foraminifers, calcispheres and oolites, the surface area used for frequency index was 110 mm^2, for detrital quartz, it was 17.9 mm^2.

The parameters occurring with sufficient frequency to be statistically valid were then plotted, using appropriate arithmetic or logarithmic scales, according to their stratigraphic position in the column. By joining these points, a series of curves were obtained which trace the stratigraphic variation of each particular parameter. The presence of components not abundant enough to allow frequency measurements was indicated by vertical lines in the appropriate column.

On the basis of microscopic textures, presence of a detrital or recrystallized matrix, average values and relationships of organic and inorganic parameters, the thin sections were divided into distinct microfacies. In the last column of each set of variation curves, these microfacies have been arranged from left to right in order of decreasing relative depth of deposition or increasing energy level. The curve resulting from this arrangement is called the relative bathymetric curve. It reflects, in this particular case, both changes in energy level and water depth, and represents the final interpretation of the environmental evolution through time of the carbonates.

Components

The limit between carbonate skeletal or mineral grains and matrix was set at 60 microns.

Crinoids occur most commonly as columnals and ambulacral fragments. A significant amount of this material displays a characteristic coarse lattice in thin section and while it may belong partly to crinoid calyxes, some of it might be derived from the plates of other echinoderms, such as blastoids and starfish. This assumption is supported by the occurrence of a substantial number of echinoid spines. All plates were entered as part of the frequency counts for crinoids, however, the spines were not.

Fenestrate bryozoans are easily identifiable when directly associated with their zooecia. However, the spiraling stock of the genus *Archimedes*, which contributes a large proportion of the bryozoan debris, occurs as large brown, fibrous fragments.

Arenaceous foraminifers are almost all endothyrids and paleotextularids.

Oolites formed mainly around crinoid and fenestrate bryozoan fragments. They average about one-half millimeter in diameter and usually have multiple concentric rings. A number of oolites show evidence of transportation such as broken or eroded rims, or second-generation rings around the remnants of reworked oolites. Wherever long fronds of bryozoan had become oolitized they were disregarded in the measurements of index of clasticity. Elongate oolites with length: width ratios greater than 2:1 were not measured.

Quartz occurs as subangular to subrounded particles, generally in the coarse silt range, associated occasionally with larger and very elongate grains.

Chert is not very common but does occur in all microfacies. It is most common in the upper portions of the Glen Dean, Vienna, and Kinkaid Formations.

Wherever possible chert specimens were also identified as to microfacies, and in some cases indices of frequency and clasticity on the coarser elements, usually crinoids were obtained.

Phosphate usually occurs as isotropic angular fragments, although a few platy particles display a fibroradiated pattern. The latter are interpreted as fish scales while the other phosphate debris probably represent broken-up conodont remains.

Description of Microfacies

Petrographic examination disclosed the presence of six distinct microfacies. One of them, a pressure-welded biocalcarenite (microfacies 3d), was found to transgress across the microfacies boundaries of all of the grain-supported rocks regardless of matrix type, and was therefore not used as a potential indicator of environment because of its obvious generation through diagenetic processes.

The remaining five microfacies form a genetically related sequence indicative of variations of conditions of deposition in a relatively quiet environment since they are either mud-supported or grain-supported with a microcrystalline calcite matrix which has recrystallized to a variable degree.

Microfacies 1.—Megascopically, microfacies 1 is a light grayish-buff to gray, sub-lithographic to very fine-grained limestone. The lighter colored, less argillaceous variety occasionally displays a slightly conchoidal fracture. Fossils, when present, are large and generally unbroken.

Microscopically, microfacies 1 (FIGURE 3A) is a calcisiltite with scattered fine sand-size organic debris and detrital minerals. The groundmass is a black microcrystalline calcite and the most frequent bioclasts are calcified monaxonic sponge spicules and small crinoid fragments. Ostracods, both as single valves or as complete individuals frequently occur but are by no means omnipresent. Brachiopod and pelecypod shells, and debris of trilobites occasionally appear as large, nontransported fragments, indicating an *in situ* community. Calcispheres, calcareous algae, bryozoans, and arenaceous foraminifers are sparse.

One or two oolites may sometimes be found but invariably they have damaged rims, indicating transportation. Detrital quartz also has a relatively low frequency of occurrence in this microfacies. Chertification occurs only occasionally, preferentially replacing sponge spicules and matrix.

A common feature of microfacies 1 is a laminated structure caused by concentration of the bioclasts along particular zones (FIGURE 3A). Post depositional disturbance, probably due to the burrowing action of scavengers, has rearranged the organic debris into spiral patterns. In one or two samples, pockets of very coarse crinoidal debris cemented by cavity-filling calcite, were found to be completely enclosed by a calcisiltite matrix and could represent fillings of burrows.

Microfacies 2.—Megascopically, microfacies 2 is a dark gray to grayish-buff, buff weathering, sub-lithographic to finely crystalline limestone. The denser varieties often display a conchoidal fracture, while the more argillaceous units show distinct laminations. This microfacies occurs in thin to massive beds, ranging from 10 cm to 1.3 meters in thickness.

Microfacies 2 is a mud-supported biocalcarenite (FIGURE 3B) and characterized by having more than 50% of the bioclasts larger than 60 microns. These are most commonly crinoid fragments and echinoid plates, calcified monaxonic sponge spicules, and bryozoans. A unique feature of this microfacies is the general presence of echinoid spines. Calcispheres, ostracods, and arenaceous foraminifers are of secondary importance, whereas debris of brachiopods, pelecypods, gastropods, trilobites, and of rodlike calcareous algae make up the remainder of the organic components. Detrital quartz of fine sand-size is accompanied by a few phosphate fragments and some abraded oolites obviously transported into this environment. Several spiral structures indicating scavenger action were observed.

Partial recrystallization of the matrix into microspar may be seen in several specimens and in a few instances the entire matrix has been changed into an equant mosaic of pseudospar (50-60 microns).

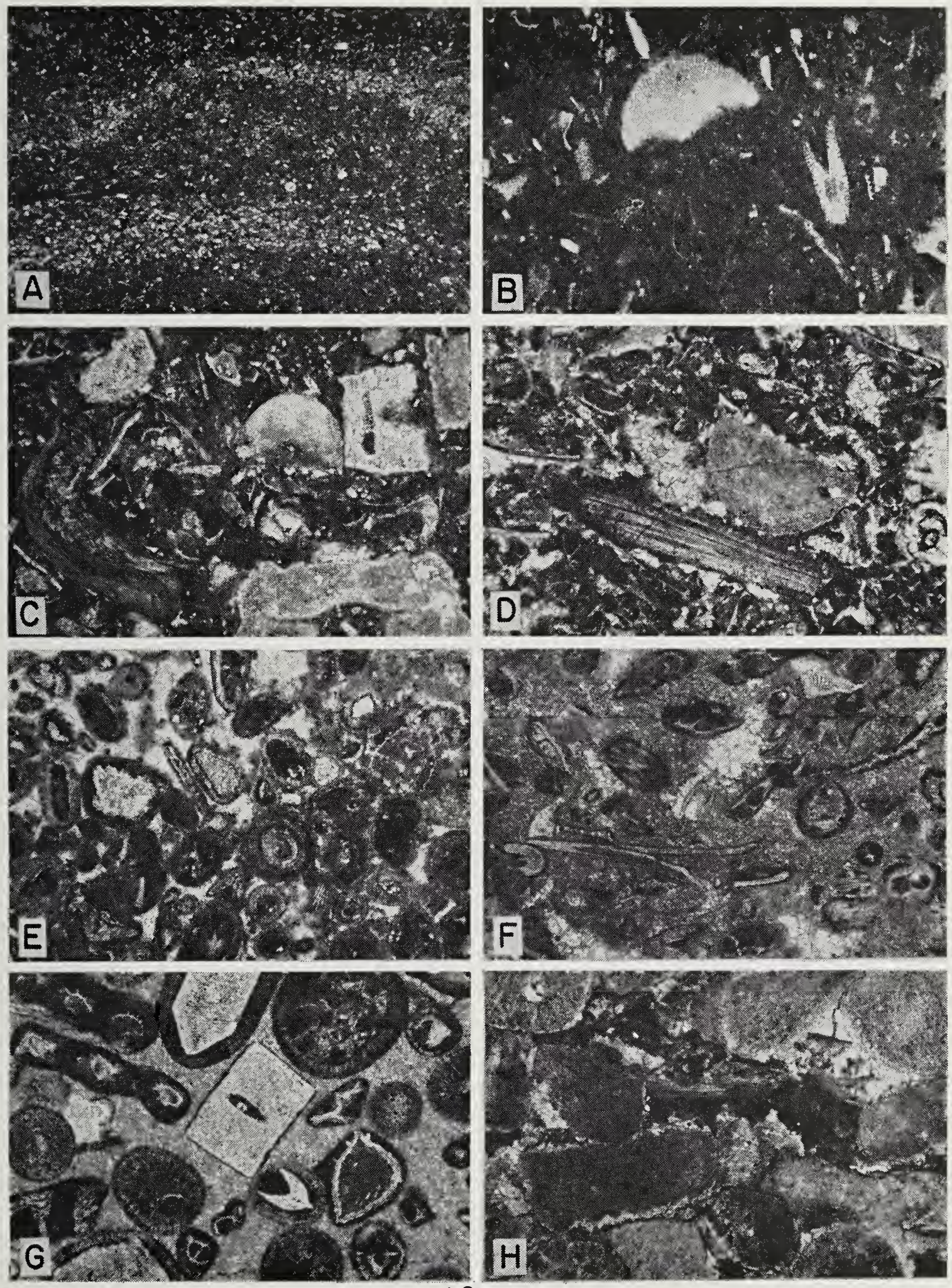
Microspar and pseudospar are types of neomorphic calcite (Folk, 1965) defined as sparry calcite larger than 3.5 microns which results from recrystallization. Microspar ranges from 3.5 microns to an arbitrary upper limit of 30 microns, while pseudospar includes all sparry calcite above this limit. This process of recrystallization will be covered later on in further detail.

Microfacies 3a.—In the field, microfacies 3a occurs as a light gray to light grayish-buff, coarsely crinoidal limestone. Large fossil fragments are frequent, however, whole shells are rare. Again, the more argillaceous members of this microfacies display a finely laminated structure. The thickness of beds ranges from a few centimeters to a meter.

Microscopically microfacies 3a (FIGURE 3C) is a grain-supported biocalcarenite with a calcisiltite matrix in which recrystallization to microspar or pseudospar does not exceed 20% of the surface. In this study the fabric was termed grain-supported when the bioclastic to matrix ratio was greater than 50%. The predominant bioclastics are crinoids and fenestrate bryozoans. Arenaceous foraminifers, calcispheres, ostracods, and calcified monaxonic sponge spicules are relatively minor constituents. Fragments of brachiopods, pelecypods, gastropods, and calcareous algae are well represented and trilobite debris are more common than in microfacies 1 and 2. Also in this microfacies echinoid spines are present in nearly every slide. Evidence of scavenger action and organic phosphate debris are rare.

Detrital quartz averages slightly more than 0.1 mm in diameter and reaches its peak in frequency in this microfacies.

A few samples contain only detrital quartz grains in a matrix of cryptocrystalline calcite. While some of them should probably be called sandstones



Typical Microfacies

FIGURE 3. — A. *Microfacies 1* (Kinkaid Formation, Type Section, Loc. No. 18) Banded calcisiltite with scattered fine sand-sized bioclastic debris, mostly crinoids, ostracods and calcified monaxonic sponge spicules. Nicols not crossed.

B. *Microfacies 2* (Menard Formation, Chester East, Loc. No. 12)
Mud-supported biocalcarenite with calcisiltite matrix. "Floating" bioclasts are largely crinoid and echinoid fragments with a few scattered debris of bryozoans and ostracods. Nicols not crossed.

C. *Microfacies 3a* (Glen Dean Formation, Reilly Lake North, Loc. No. 9)
Grain-supported biocalcarenite with calcisiltite matrix, consisting mostly of large fragments of crinoids and bryozoans. Arrangement of debris suggests post-depositional disturbance by scavengers. Note fibrous debris of *Archimedes* in lower left corner. Nicols not crossed.

D. *Microfacies 3b* (Kinkaid Formation, Type Section, Loc. No. 18)
Grain-supported biocalcarenite with calcisiltite matrix partially recrystallized into pseudospar. Bryozoan debris predominant. Large linear element at lower center is a portion of spiraling stock of *Archimedes*. Nicols not crossed.

E. *Microfacies 3b* (Menard Formation, Chester East, Loc. No. 12)
Grain-supported oolitic biocalcarenite with calcisiltite matrix partially recrystallized to pseudospar (particularly in upper left corner). Note poor sorting of transported oolites and intercalations of black microcrystalline calcite between concentric rings. Nicols not crossed.

F. *Microfacies 3c* (Glen Dean Formation, Reilly Lake North, Loc. No. 9)
Grain-supported biocalcarenite with secondary cement of dominantly microspar with patches of pseudospar. Predominant bryozoan zooecia filled with and surrounded by dark microcrystalline calcite. Nicols not crossed.

G. *Microfacies 3c* (Glen Dean Formation, Reilly Lake North, Loc. No. 9)
Grain-supported oolitic biocalcarenite with secondary cement of pseudospar. Note irregular size distribution of transported oolites and bioclasts with rims of microcrystalline calcite. Nicols not crossed.

H. *Microfacies 3d* (Menard Formation, Chester East, Loc. No. 12)
Pressure-welded crinoid-bryozoan calcarenite. Note deep reciprocal interpenetration of all elements which are aligned parallel to bedding, and distortion of more fragile fenestrate bryozoans. Nicols not crossed.

with a calcareous cement they were nevertheless placed in this class.

Microfacies 3b. — Megascopically, microfacies 3b differs from 3a only in having a more coarsely crystalline aspect. In some places the fossil fragments are concentrated along definite zones giving a bedded or sometimes even cross-bedded aspect to the rock. When these conditions occur, the individual beds are 0.5 to 1.0 meters thick. However, there is considerable variation in thickness within the units representative of microfacies 3b.

Microfacies 3b (FIGURE 3D) is a grain-supported biocalcarenite with a calcisiltite matrix showing 20 to 80% of its surface recrystallized to microspar or pseudospar. The dominant bioclastics, showing varying degrees of pressure-solution, still consist principally of crinoids and fenestrate bryozoans with the proportion of crinoids to bryozoans decreasing.

Ostracods, calcified monaxonic sponge spicules, arenaceous foraminifers, and calcispheres are less abundant than in the preceding microfacies. Debris of brachiopods, pelecypods, gastropods, trilobites, calcareous algae, and echinoid spines are minor constituents as are detrital quartz and organic phosphate debris. Worm tubes and disruption by scavengers are rare. Oolites become more important in microfacies 3b, occurring generally in well packed to slightly pressure welded arrangements (FIGURE 3E). The oolite cores are mainly bryozoan fragments, with crinoids and other organic debris being of secondary importance. These oolites are apparently transported, but where the

surrounding microcrystalline matrix has been recrystallized, their edges have become further indented by this process.

Microfacies 3c.—Microfacies 3c is the most easily recognizable of the five lithologies in the field. It is usually light gray or light buff in color, sometimes changing to dark gray in the more finely crystalline varieties. The lighter, more common members of this class contain abundant large, well preserved fossils, usually broken, although whole specimens are not uncommon. Microfacies 3c is often oolitic and cross-bedded. Beds are normally 0.5 to 1.5 meters in thickness, although they are somewhat thinner in a few places.

In the most characteristic examples of this class the bioclasts seem to "float" in a glass-like groundmass of pure sparry calcite cement.

Under the microscope, microfacies 3c is a grain-supported biocalcarenite in which more than 80% of the original calcisiltite matrix has recrystallized to microspar and pseudospar. Cavity-filling sparry calcite is absent in this microfacies. The neomorphic calcite displays the following varieties: microspar with scattered patches of pseudospar (FIGURE 3F); pseudospar with smaller areas of microspar; fine or coarse uniform pseudospar (FIGURE 3G).

Crinoids and fenestrate bryozoan fragments dominate the organic assemblage, with bryozoans occurring with the highest frequency (FIGURE 3F). Oolites of transported nature occur at their highest frequency and clasticity, averaging over 0.6 mm in size. Most are normal oolites, consisting of either bryozoan or crinoidal debris surrounded by multiple concentric rings of fibroradiated calcite. Transportation, which is shown by poor sorting and wide ranges of size, is confirmed by the occurrence of abraded rims, intercalation of calcisiltite between concentric rings, and by excentric rings (Carozzi, 1964).

Ostracods, calcispheres and arenaceous foraminifers are rare. Brachiopod, pelecypod, gastropod, and trilobite fragments, as well as calcareous algae, echinoid spines, and organic phosphate debris make up the remainder of the assemblage.

Quartz averages 0.065 mm and is at its lowest frequency relative to the other microfacies. It is usually sub-angular to sub-rounded.

The grain-supported microfacies 3a, 3b, and 3c have been distinguished on the basis of an increasing percentage of recrystallization of the original interstitial calcisiltite matrix. In terms of energy level and general environmental interpretation it is of fundamental importance to separate low energy calcarenites with a recrystallized calcisiltite matrix from high energy carbonates with cavity-filling calcite cement. Neomorphic clear calcite resulting from recrystallization of an original calcisiltite matrix displays the following features (Bathurst, 1958; Folk, 1965): grain size ranges continuously from four microns upward; the grain size varies irregularly from place to place within the mosaic; grain boundaries are curved, consertal, and often plicate, as opposed to the straight, planar boundaries of precipitated sparry calcite. The microcrystalline matrix often displays a "nibbled" appearance, often to the point of bearing delicate stringers and patches of the original matrix completely surrounded by coarse calcite (FIGURE 3E).

Microfacies 3d.—Megascopically this microfacies appears as a very coarsely crystalline crinoidal limestone of variable color, ranging from light buff to dark gray. In some measured sections its occurrence corresponds to zones of stylolitization.

Microscopically a specimen was termed to be pressure-welded when 50% or more of the bioclastic grains were in reciprocal contact with each other and exhibited mutual interpenetration along their boundaries. All gradations of pressure-welding were noted from incipient grain contact to deep interpenetration accompanied by elimination of the original calcisiltite matrix (FIGURE 3H). Most commonly the bioclasts in these specimens consist of crinoids and bryozoans oriented parallel to bedding by the effects of pressure solution. Most of the other types of organic debris seen in microfacies 3a through 3c, with the exception of brachiopods and pelecypods, have been largely eliminated in the more advanced stages of pressure welding. Fragments of organic phosphate and grains of detrital quartz are also seen occasionally. Oolites are common in this group, although the disruption of their rims due to this process is not to be confused with changes wrought by transportation.

COMPOSITE SECTION

The five microfacies 1 through 3c described in the previous section are

gradational vertically into one another (and also horizontally according to Walther's rule) and the variation of their characteristic average parameters (TABLE 1) indicates that such a succession expresses an increase of energy level and at the same time a decrease in the relative depth of deposition. Therefore, it is possible to superpose them in a composite section or statistically most frequent succession which may be used for the interpretation of the investigated sections (FIGURE 4).

The average parameter variations of the composite section may be described as follows:

The frequency curve of crinoid debris has a symmetrical, almost bell-shaped aspect ranging from an average of 56 in microfacies 1 to a peak of 239 in microfacies 3a, then decreases to a value of 97 in microfacies 3c. The clasticity curve of crinoid debris increases linearly from 0.797 mm in microfacies 1 to a maximum value of 1.434 mm in microfacies 3c. An abrupt increase in frequency would be expected during the transition from a mud-supported rock (microfacies 1 and 2) into a grain-supported fabric (microfacies 3a). The reduction of the frequency into microfacies 3c may be explained by an increase in the sorting action of the currents or of the agitation of the environment removing smaller fragments, thereby concentrating relatively larger ones in the site of deposition, as shown by the maximum clasticity of the crinoid debris in the same terminal microfacies. Furthermore, many of the bioclastic fragments found in microfacies 3c, especially the crinoids, still display thin, discontinuous coatings of dark calcisiltitic mud indicating moderate intraformational processes of reworking. It may therefore be concluded that the variation of the crinoid parameters ex-

FIGURE 4. — Composite section

TABLE 1.—Average Parameter Values by Microfacies.

	Microfacies				
	1	2	3a	3b	3c
Crinoid Frequency	56	97	239	149	97
Crinoid Clasticity (mm)	0.797	1.109	1.397	1.354	1.434
Bryozoan Frequency	33	44	100	109	132
Arenaceous Foraminifer Frequency	24	33	14	40	9
Ostracod Frequency	24	17	11	10	3
Calcisphere Frequency	13	11	12	18	4
Sponge Spicule Frequency	1749	517	337	97	13
Detrital Quartz Frequency	11	23	31	22	3
Detrital Quartz Clasticity (mm)	0.053	0.094	0.112	0.099	0.065
Oolite Frequency	1	11	3	98	121
Oolite Clasticity (mm)	0.504	0.504	0.427	0.583	0.660
Percentage of Total Thickness	18	10	11	45	16

presses the growth of an almost autochthonous population and the local sorting of its skeletal debris.

The bryozoan frequency increases from microfacies 1, where it has a value of 33, up to 132 in microfacies 3c. There is a sharp change in the slope of this curve in passing from microfacies 2 (mud-supported) to microfacies 3a (grain-supported) which is almost parallel to the rate of crinoid frequency increase in this interval. Beyond microfacies 3a, however, bryozoan frequency increases linearly to a maximum value in microfacies 3c. This is interpreted as being an expression of the increased disintegration of the delicate bryozoan fronds with increasing energy in the environment, whereas resistant crinoid columnals were only sorted according to size but not broken.

The frequency of arenaceous foraminifers exhibits a succession of peaks and lows which do not seem to be of particular significance. The lowest frequency is found in microfacies 3c, where there are but nine foraminifers per unit area. These benthonic and rather small components have been well preserved in the mud-supported microfacies 1 and 2. Above this, their variable frequency and particularly the minimum in microfacies 3c certainly result from the effect of recrystallization processes.

The ostracod frequency decreases rapidly from a high of 24 in microfacies 1 to a value of 11 in microfacies 3a. From this point there is a more gradual decline to a low value of three counts per section in microfacies 3c. Although no distinction was made between ostracods with both valves intact and those represented by a single valve, the bivalved remains were more prevalent in the lower energy microfacies.

The frequency of calcispheres varies almost as a straight line through the first three microfacies. There is an abrupt increase from 12 to 18 calcispheres per section in passing from microfacies 3a to 3b, and just as abrupt a decrease to a low of four in microfacies 3c.

Sponge spicule frequency decreases in a linear fashion from a high of 1749 in microfacies 1 to a value of 337 in microfacies 3a. Beyond this there is a

sharp change of slope in the curve and the spicule frequency drops off quite rapidly to a value of only 13 spicules per section in microfacies 3c. This curve expresses the liberation in place of sponge spicules and indicates that siliceous sponges reach here their best development in the fine-grained carbonates deposited in quiet and relatively deep conditions.

There is a similarity in behavior between the frequency curve of sponge spicules and that of benthonic arenaceous foraminifers and ostracods and of pseudopelagic calcispheres. All these curves display a general decrease in frequency upwards which may be linear or oscillatory. Actually arenaceous foraminifers, ostracods and calcispheres which should have been present throughout the spectrum of the microfacies have been predominantly preserved only in the low energy conditions of the mud-supported microfacies 1 and 2 and were largely winnowed from the more agitated environment of the grain-supported microfacies 3a, 3b and 3c where only a small number of them could remain caught between larger bioclasts. These somewhat more agitated conditions also explain the higher frequency of single ostracod valves as opposed to the greater abundance of whole individuals in the lower energy microfacies. Similar conditions have been reported from the Rundle Group (Mississippian) of the Front Ranges in Central Alberta, Canada (Walpole and Carozzi, 1961).

Furthermore, the minimum values for the preceding parameters in microfacies 3c may also be ascribed to the additional destructive effect of recrystallization of the matrix.

Detrital quartz shows a symmetrical behavior of both clasticity and frequency, indicating by their parallelism that the supply was regular but never very abundant (Carozzi, 1958). In quartz grains ranging in size from coarse silt to fine sand (0.053 mm to 0.112 mm), it is natural that the larger and more abundant particles settle in microfacies 3a, as an effect of winnowing in the higher energy environments of 3b and 3c. Only smaller and less abundant particles settled in the mud-supported microfacies.

Oolite frequencies begin with low values of 1, 11, and 3 counts per section through microfacies 1, 2, and 3a respectively. The frequency increases abruptly from microfacies 3a to 3b, reaching a value of 98 at the latter level, thence to a maximum of 121 in microfacies 3c. Oolite clasticity follows the frequency curve quite closely and similarly increases abruptly from 0.427 mm in microfacies 3a to a maximum of 0.660 mm in microfacies 3c. The occurrence of broken oolites and of others displaying internal residual layers of dark calcisiltite mud (Carozzi, 1964) indicate that the oolites are essentially transported and originate in environments of relatively high energy adjacent to microfacies 3a-3c. Therefore, they should be interpreted as detrital components distributed from shallow into deep conditions and because of their size, rather uneffected by winnowing.

The final interpretation of the five microfacies in terms of increasing relative energy level or decreasing relative depth is plotted as a smooth curve showing a steady increase in energy from 1 through 3c.

Environmental Interpretation

The horizontal reconstruction of this low energy environment of carbonate deposition submitted to moderate winnowing processes, shows three major subdivisions (FIGURE 5).

The first one consists of microfacies 1 and 2 which are similar except for the ratio of bioclastic debris to matrix being higher in microfacies 2. They represent extremely quiet conditions characterized by the deposition of fine-grained carbonate mud in which siliceous sponges and scavengers were very abundant among scattered clumps of crinoids. As an effect of winnowing processes taking place in higher energy and shallower conditions arenaceous foraminifers, ostracods and calcispheres were finally deposited here associated with a small number of detrital quartz grains, transported oolites and bryozoan debris.

The second subdivision is represented by microfacies 3a, the first

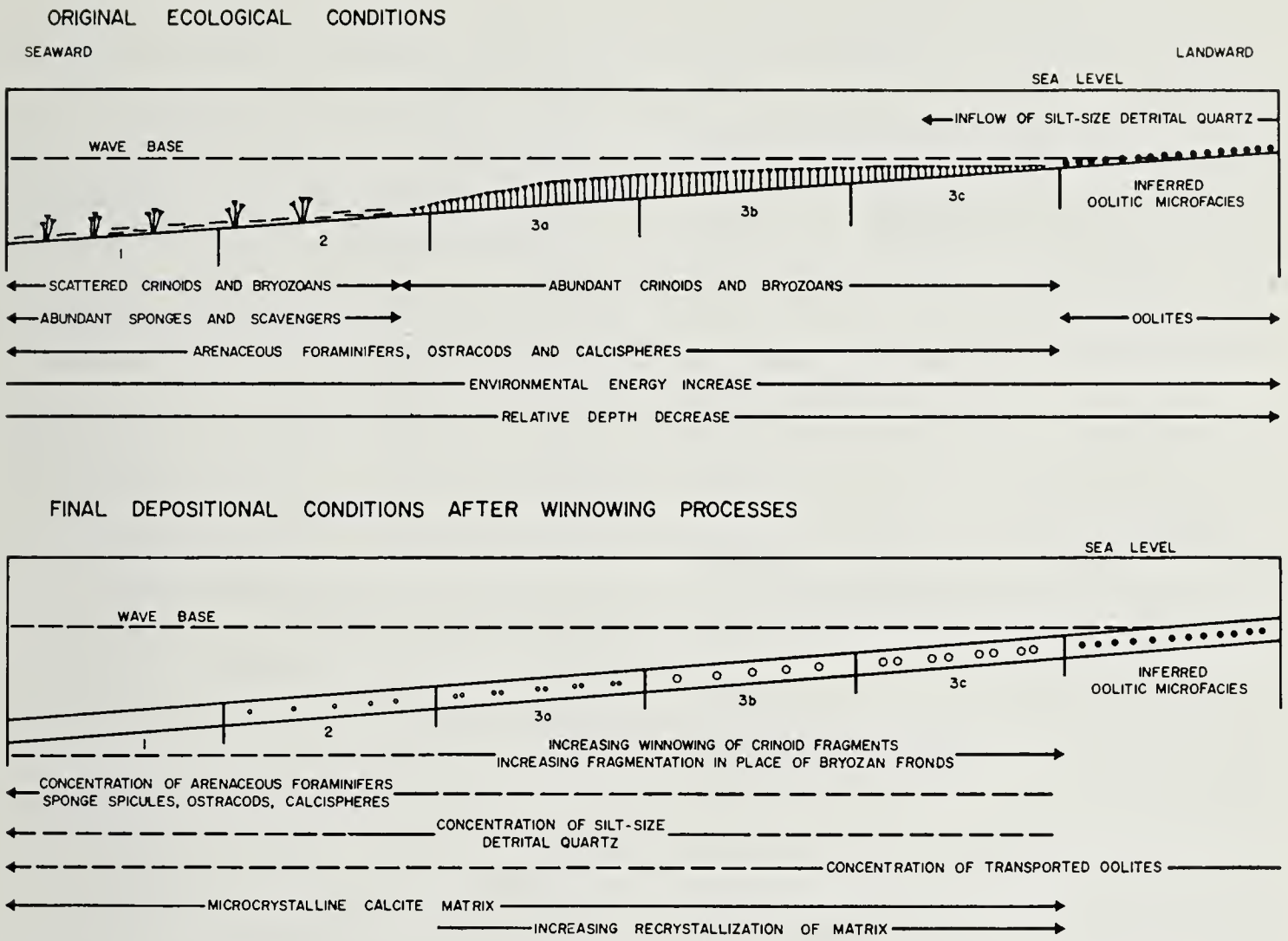


FIGURE 5. — Environmental interpretation

belonging to the grain-supported group characterized by a large development of crinoid and bryozoan colonies. It is in this intermediate position that most of the silt-size detrital quartz of extrabasinal origin, winnowed from higher energy conditions, was finally deposited.

The third subdivision consists of microfacies 3b and 3c and corresponds to higher relative energy and shallower conditions, although an even shallower environment must be inferred from which abundant transported oolites originated. The winnowing conditions which characterize these two microfacies have sorted the crinoid debris, fragmented the delicate bryozoan fronds and almost completely eliminated the silt-size detrital quartz and the small-size organic components such as the arenaceous foraminifers, ostracods and calcispheres.

Although the group of grain-supported microfacies shows an increase in the degree of diagenetic recrystallization of the original calcisiltite matrix into neomorphic calcite apparently related to an increase in the energy level or a decreasing relative depth, such a relationship may be entirely fortuitous.

Description of Sections

In the following discussion of the individual measured sections reference will be made to the respective sets of variation curves (FIGURES 6-11). Since the microfacies parameters have already been discussed in the composite section in some detail, attention here will be directed to the unusual features of each unit. A

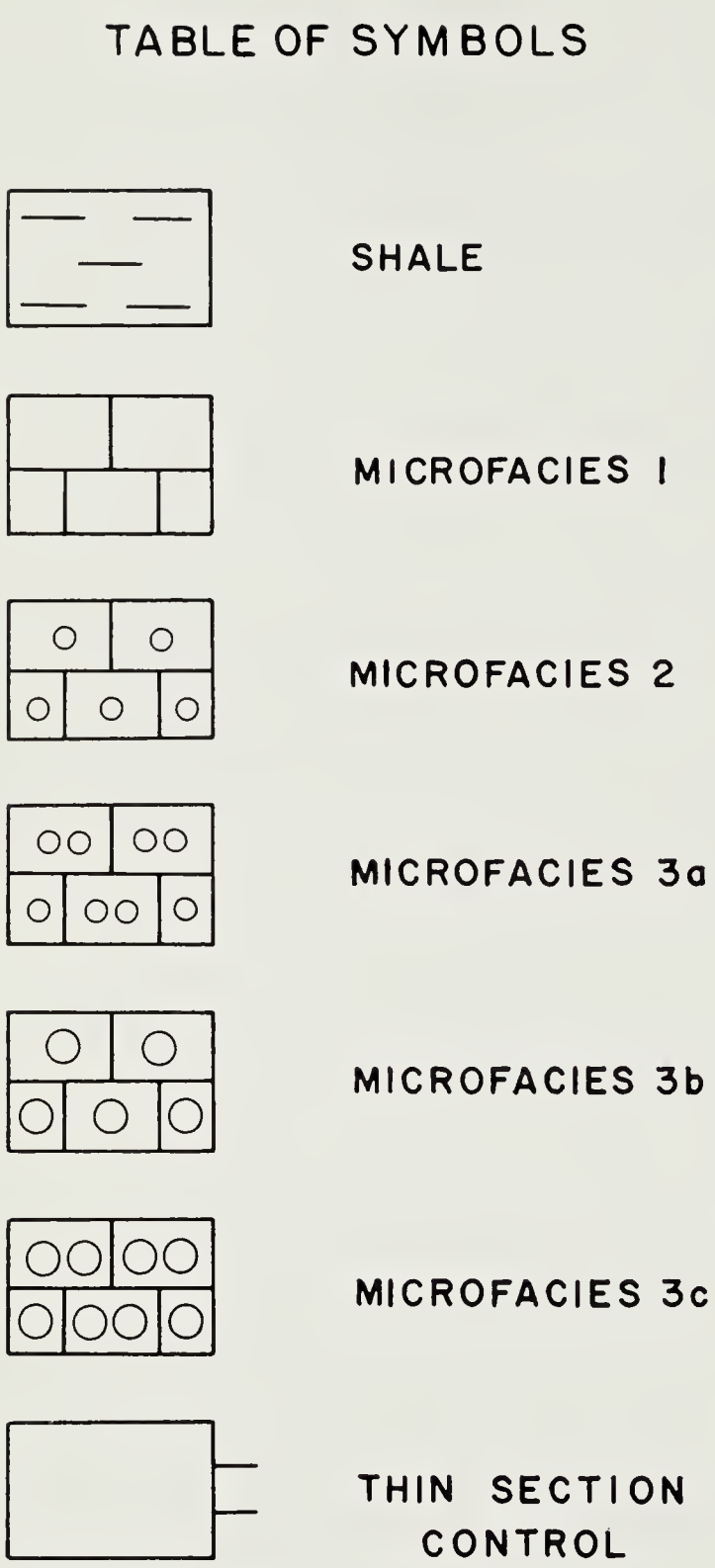


FIGURE 6.—Table of symbols

smooth main trend curve has been superimposed as a dashed line on the relative bathymetric curve in order to show the general evolution of the sedimentation which displays a definite cyclicity within a low energy environment.

Section No. 9, Reilly Lake North. —Section of Glen Dean Formation exposed in small quarries in Mississippi Valley bluff about 1.1 miles northwest of Reilly Lake, SE¼, SE¼ sec. 24 (extended), T. 6S., R. 8W., Randolph Co., Illinois.

The lower part of this section (FIGURE 7) is characterized by a marked parallelism of crinoid and bryozoan frequencies. The oolite frequency is opposed to the preceding parameters as is to be expected, since an abundance of the former components precludes the presence of the latter. Crinoid and oolite clasticities are also quite similar. Most of this part of the section is in microfacies 3c although it does drop into 3b in a few places.

The upper portion of this section shows considerably more variation, mainly between microfacies 3a and 3b but occasionally reaching microfacies 1. Crinoid and bryozoan frequencies are again parallel with the exception of opposed curves near 14 meters. Benthonic ostracods and pseudopelagic calcispheres exhibit frequency curves which are opposed to those of the crinoids and bryozoans, thereby indicating their susceptibility to winnowing. Associated with the lower energies, detrital quartz appears in this section, with highest frequencies in microfacies 1 and 2.

Section No. 10, Coles Mill North. —Upper Glen Dean Formation exposed along railroad tracks behind flour mill at south edge of Chester, W. line NW¼ sec. 30. T. 7S., R. 6W., Randolph Co., Illinois.

As in the preceding section the environment is one of relatively high energy with the main trend fluctuating only slightly between microfacies 3b and 3c (FIGURE 7). Parallel trends of crinoid and bryozoan frequency, and of crinoid and

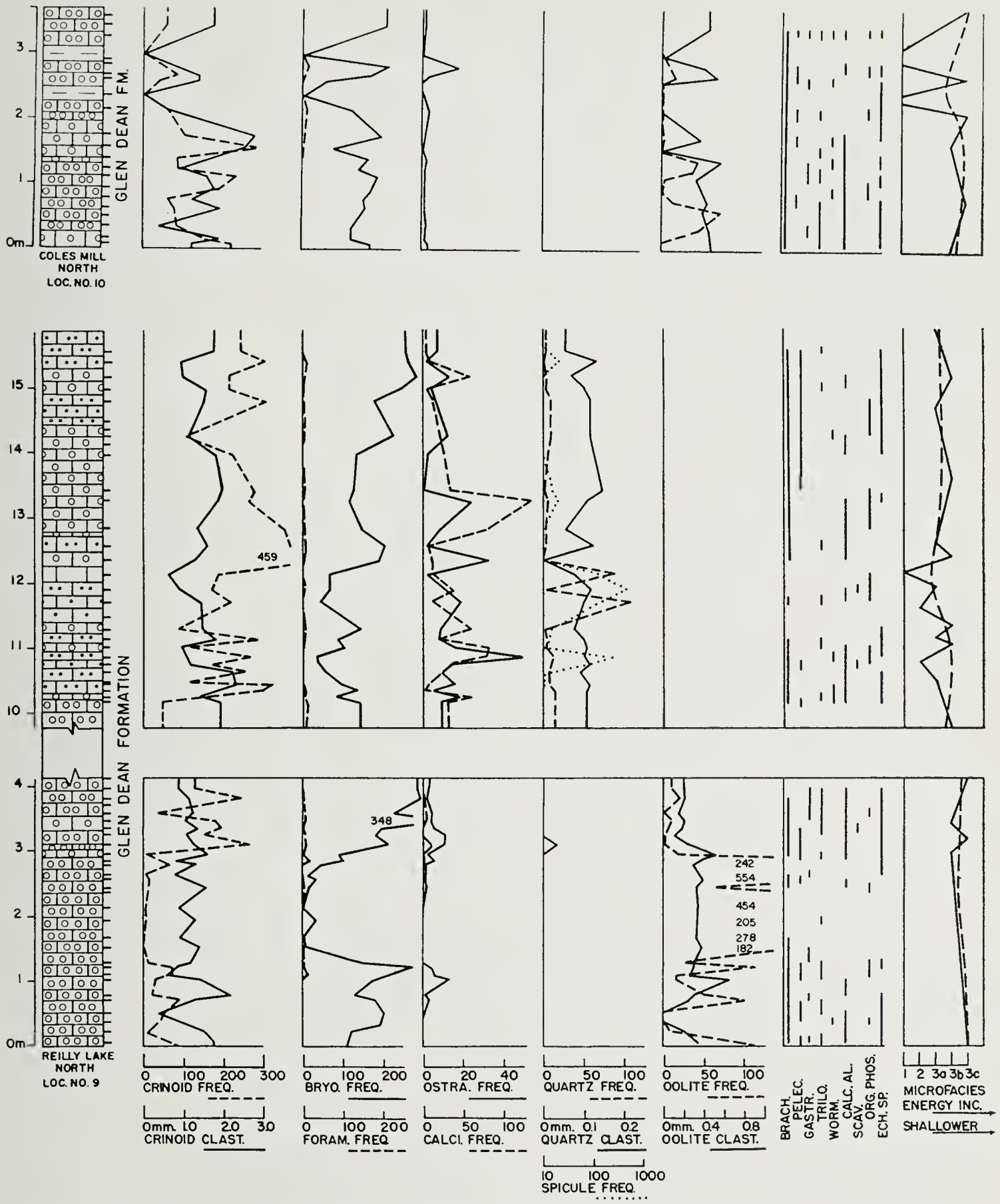


FIGURE 7. — Reilly Lake North, Locality No. 9, and Coles Mill North, Locality No. 10.

oolite clasticity are obvious. Oolite frequency opposes those of the crinoids and bryozoans.

Section No. 10A, Coles Mill South. —Vienna Formation exposed in road cut about 200 yards south of section No. 10, NW¼ NW¼ sec. 30, T. 7S., R. 6W., Randolph Co., Illinois.

The general trend curve (FIGURE 8) shows the upper and lower portions of this section to vary between microfacies 3a and 3b while

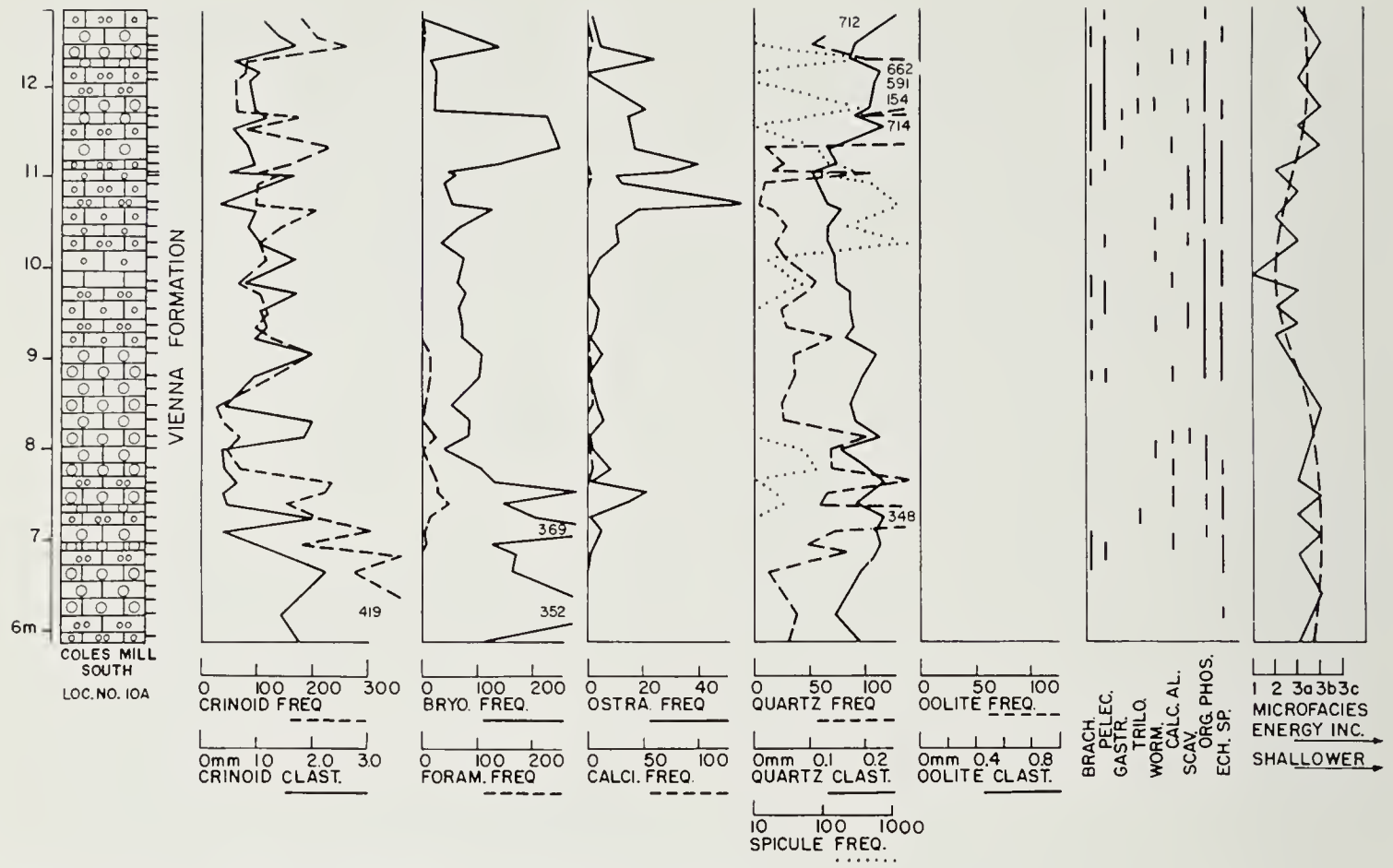


FIGURE 8. — Coles Mill South, Locality No. 10A

in the middle, between 3 and 5 meters, it reflects somewhat lower energy conditions. The frequency curves of crinoids and bryozoans, as well as that of detrital quartz, exhibit the same parallelism noted in the previous two sections. Ostracod frequency runs opposed to the peaks of the crinoid and bryozoan frequencies, again as an expression of the winnowing of small size components from high energy environments. Detrital quartz clasticity closely follows the peaks and lows of its frequency.

Section No. 12, Chester East. — Menard Formation exposed in abandoned high level quarry in Mississippi River bluff opposite sand and gravel docks, about 1.4 miles above mouth of Marys River, SW¼ SE¼ sec. 30, T. 7S., R. 6W., Randolph Co., Illinois.

This section is broken into four parts by three 1.5 to 2.0 meter thick shales (FIGURES 9, 10). The lowermost portion is characterized by relatively high energy as shown by microfacies 3b and 3c. These conditions are again reflected in the upper portion of the section. The longest uninterrupted part of the section (5 to 14 meters) consists of a series of symmetrical fluctuations ranging from microfacies 1 to 3c. Relatively quiet conditions prevailed after the deposition of the overlying shale, as microfacies 1 is predominant.

Bryozoan and crinoid frequencies parallel each other, although the similarities of their curves are not as striking as in previous sections. The frequency curves of ostracods, arenaceous foraminifers, sponge spicules, and to a certain extent, calcispheres, show some parallelism in their trends, and are generally opposed to those of crinoids, bryozoans, and oolites. This situation again expresses the effects of winnowing.

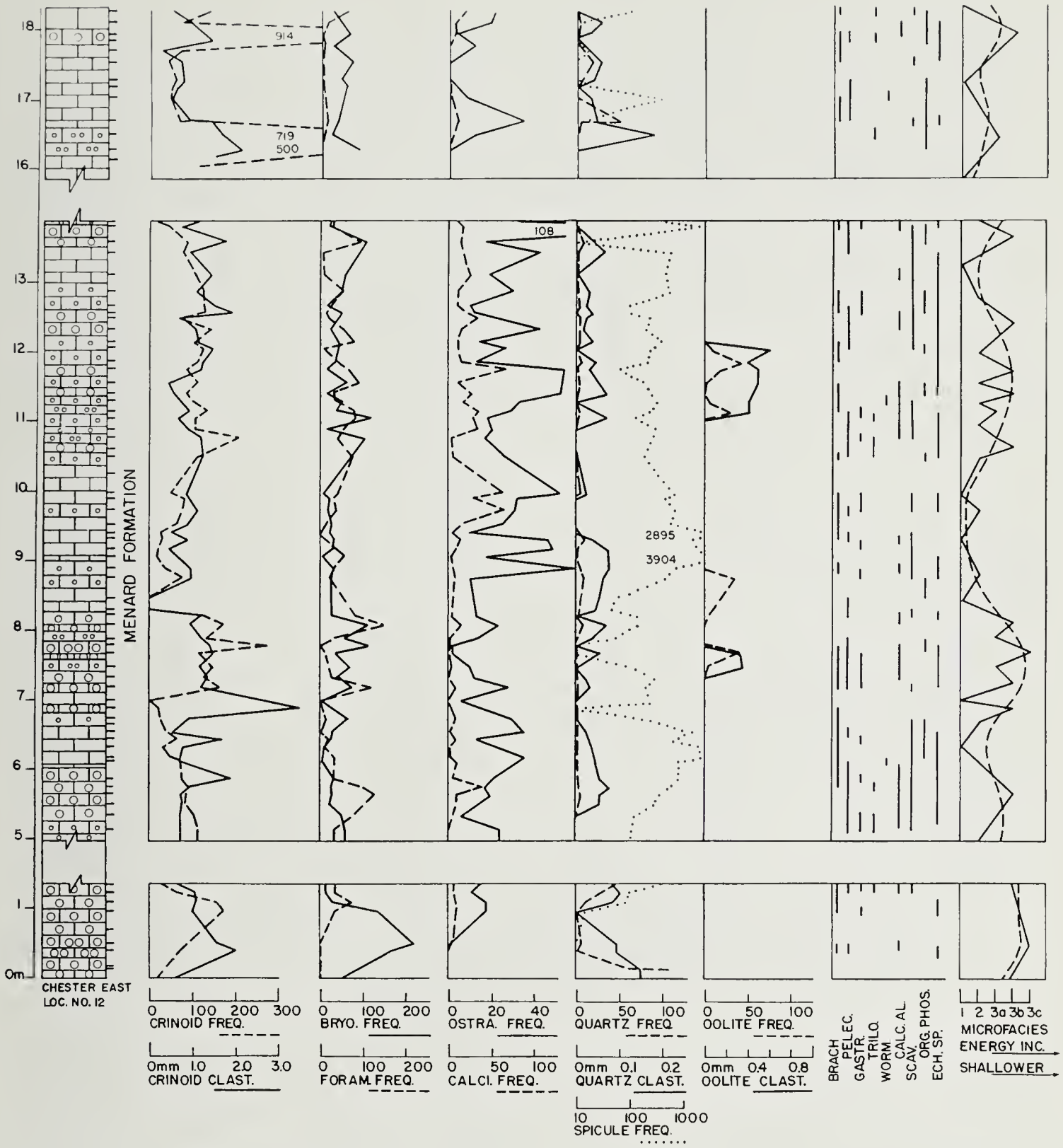


FIGURE 9. — Chester East, Locality No. 12

Section No. 16, Ford W. P. A. Quarry.—Clore Formation in small abandoned quarry in Mississippi River bluff above state highway 3, about 1.3 miles southeast of Marys River bridge, NW¼ SE¼ sec. 33, T. 7S., R. 6W., Randolph Co., Illinois.

This section begins and ends in low energy conditions but rises to a peak in microfacies 3b near its middle (FIGURE 10). Crinoid and bryozoan frequencies generally parallel each other while the frequencies of arenaceous foraminifers and ostracods are generally opposed to them. Sponge spicules and detrital quartz are quite abundant throughout most of the section.

Section 18, Type Kinkaid.—Type Kinkaid Formation on north bank of Kinkaid Creek in Illinois Stone quar-

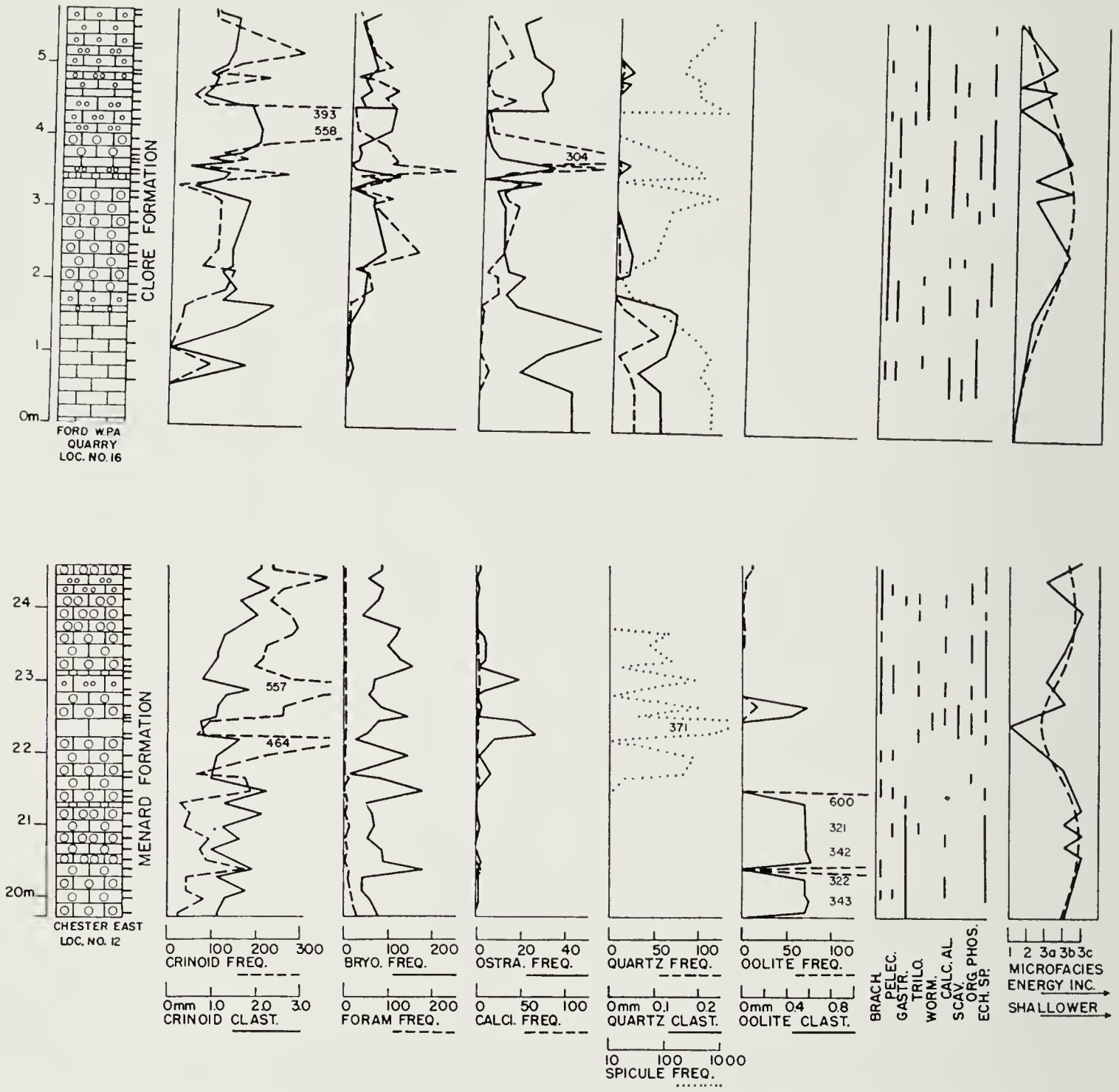


FIGURE 10. — Chester East, Locality No. 12, and Ford W. P. A. Quarry, Locality No. 16.

ry, SE¼ NW¼ NW¼ sec. 6, T. 8S., R. 4W., Jackson Co., Illinois.

The Kinkaid Formation (FIGURE 11) is divided into an upper and a lower unit by a persistent 0.7 to 2.0 meter bed of shale with interbedded limestone stringers. The lower part exhibits the distinctive crinoid-bryozoan parallelism, whereas this frequent relationship disappears in the upper half, mainly due to an almost complete loss of bryozoan debris in the upper 3 or 4 meters. The lower half of this section, and the lower 5 meters of the upper section are characterized by several long-period, fairly symmetrical fluctuations of the environment, the major portion of the column being represented mainly by microfacies 3a, 3b, and 3c. The upper 3 meters of the Kinkaid are entirely in microfacies 1 and 2 and are particularly marked by an intense development of sponge spicules.

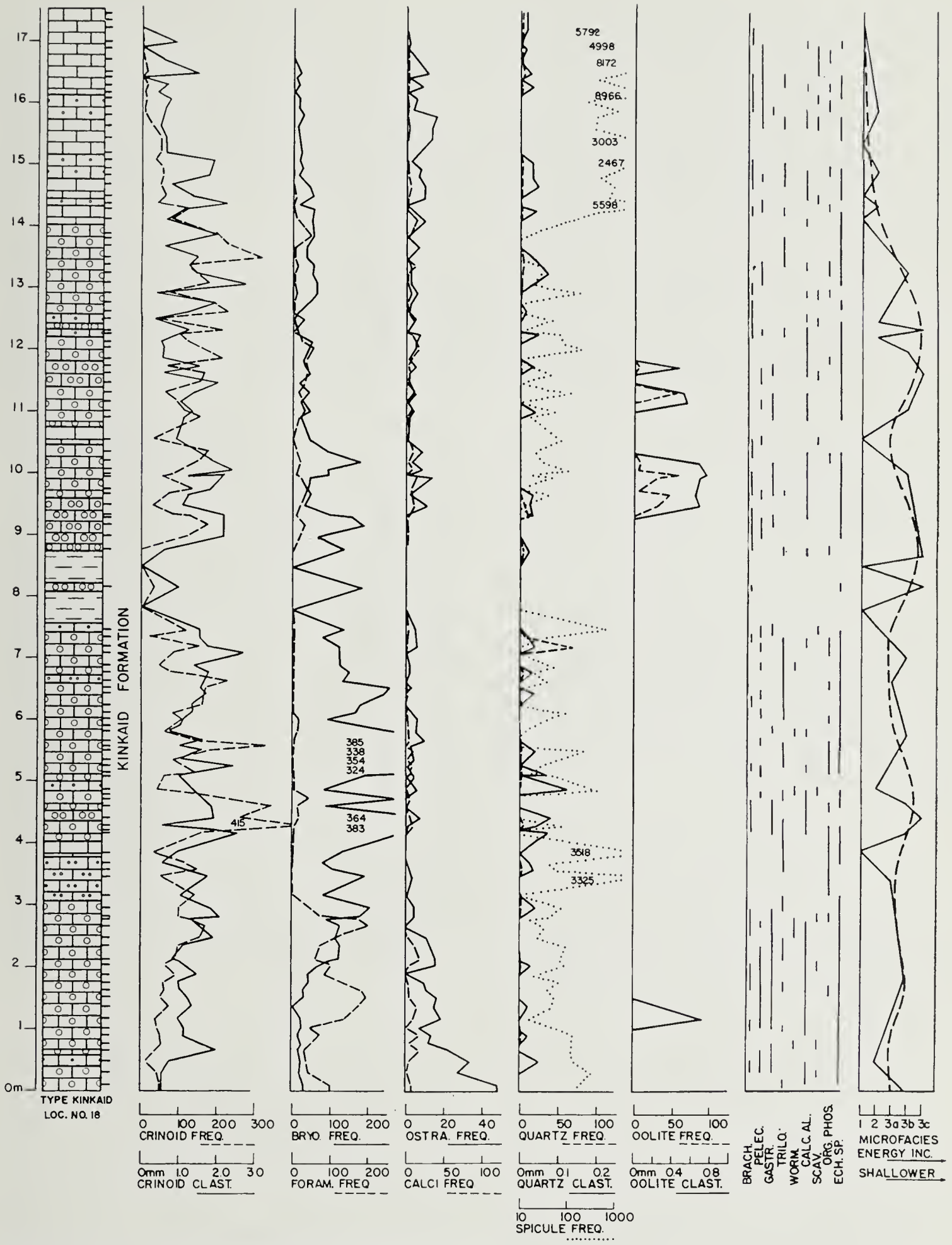


FIGURE 11. — Type Kinkaid, Locality No. 18

CONCLUSIONS

The carbonates of the upper half of the Chester Series in the type area of Southwestern Illinois (Glen Dean Formation to Kinkaid Formation) consist of an association, at times regularly cyclical, of five distinct and environmentally controlled micro-

facies. These range from calcisiltite through mud-supported biocalcarenite to grain-supported biocalcarenite with calcisiltite matrix in order of increasing energy level or decreasing relative depth. All these microfacies indicate an environment of general low energy in which moderate winnowing processes were active. In the shallower portions where crinoid and bryozoan colonies thrived, they led to the inflow of transported oolites, the sorting of the crinoid debris and the fragmentation of the delicate bryozoan fronds almost in place. In the deeper portions of the environment where abundant siliceous sponges and scavengers were associated with small crinoid clumps, the winnowing processes concentrated the silt-size detrital quartz and all the small organic components such as arenaceous foraminifers, ostracods and calcispheres originally distributed thoughout the environment.

Acknowledgments

The authors are grateful to Dr. D. H. Swann of the Illinois State Geological Survey for his interest in the investigated problem and stimulating discussions.

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Manuscript received July 17, 1967.

NOTES

ALKALOID PRODUCTION IN HEALTHY AND AMPELAMUS-VIRUS INFECTED PERIWINKLE, VINCA ROSEA L.

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If the biochemical syndrome of virus-infected plants differs from the biochemistry of noninfected plants, detection of this difference should be ascertainable with modern techniques. Since many of the hydrolytic products of plant viruses contain nitrogen, it seems likely that viral infections may induce aberrations in the plant's nitrogen metabolism and thus influence the synthesis, assimilation, or accumulation of organic bases such as alkaloids. Since Ampelamus virus invades periwinkle, *Vinca rosea* L., systemically (unpublished data) and since some new alkaloids with antitumor activity have been isolated from periwinkle (Johnson et al., 1960; Neuss et al., 1959; Noble et al., 1958; Svoboda et al., 1959) it seemed worthwhile to compare the production of alkaloids in healthy and Ampelamus-virus infected periwinkle plants. The findings are reported and discussed.

Materials and Methods

Ampelamus virus used in the experiments was isolated from *Ampelamus albidus* (Nutt.) Britt. (honeyvine, bluevine, or sandvine) in 1958 and maintained in *Nicotiana glutinosa* L. The virus, probably a strain of cucumber mosaic virus (*Marmor cucumeris* H.) is tentatively designated a distinct virus awaiting a more accurate identification.

Periwinkle plants, healthy and infected, were a single clone which had been propagated vegetatively since 1956. Infected plants were increased from a single plant of the clone inoculated mechanically. The healthy and infected plants were started in potted soil in a glasshouse and transferred May 21, with the potted soil, to field plots reasonably uniform in fertility, texture, and topography. The plants were harvested October 20.

Results and Discussion

Drying of plant tissues. Samples of periwinkle leaves and roots from healthy and virus infected plants were dehydrated in a vacuum desiccator over potassium hydroxide pellets at room temperature. Three samples of healthy leaves (each 200 grams wet weight) dried to 52, 67, and 32 grams. Four samples of diseased leaves (each 200 grams wet weight) dried to 53, 57, 50, and 59 grams. Two samples of healthy roots (250 and 290 grams wet weight) dried to 55 and 65 grams, respectively. Two samples of diseased roots (206 and 190 grams wet weight) dried to 45 and 43 grams, respectively.

Extraction of alkaloids. The dry-weighed sample of healthy or diseased leaves or roots was blended in a commercial blendor for five minutes with a mixture of 50 ml benzene, 25 ml ethanol, and 1 ml of NH_4OH. After blending, the slurry was transferred to a beaker and stirred for six hours with 200 ml of the above mixture. The liquid was filtered through paper, and the residue was extracted overnight with 200 ml of fresh solvent mixture. The liquid of the second extraction was filtered and combined with the filtrate from the first extraction. After adding 100 ml of 10% acetic acid to the combined filtrates, the liquid was concentrated by evaporation until no organic solvent remained. The

acetic acid solution was alkalized with NH_4OH and extracted with methylene chloride. Alkaloids were obtained from the dried and evaporated solution.

Analysis of alkaloids. The yield of alkaloids was in each case 0.8%± 0.2%. Thin layer chromatography of these fractions on a silica plate containing 100 μg per application showed no qualitative or quantitative difference after developing in a solvent mixture of 3 parts ethyl acetate and 1 part ethanol and spraying with ceric ammonium sulfate in phosphoric acid (Cone, Miller, and Neuss, 1963).

These findings indicate that the production of alkaloids by periwinkle is not grossly, if at all, influenced by Ampelamus-virus infection under the conditions of this experiment. It is conceivable, however, that under other conditions alkaloid production or accumulation in periwinkle might be influenced by Ampelamus-virus infection or by infection by other viruses. Although there was no detectable difference in alkaloid content of healthy and infected periwinkle, the infection may have actually influenced the metabolic pathways involved in alkaloid synthesis or utilization but allowed the same amount of alkaloid to accumulate. From these findings, it seems unlikely that alkaloid production in periwinkle is associated with metabolism influenced by infection with Ampelamus-virus. The findings orient research in other directions.

Acknowledgments

The authors are grateful to Dr. M. Gorman, Chemical Research Division, Eli Lilly Company for arrangements in his laboratory for the chemical work on the alkaloids.

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Manuscript received November 23, 1967.

BACTERIAL POPULATION OF GROSSLY HEALTHLY FROGS

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ABSTRACT.—A study was undertaken to ascertain the bacterial flora existing in apparently healthy frogs (*Rana pipiens*) of both sexes and at all times of year. The frogs were obtained from several commercial sources in the North-Central and Eastern parts of the United States. After one week's isolation, the frogs selected were those without symptomatology. Cultures of the blood, peritoneum and viscera (excluding the stomach and intestines) were found to be bacteria-free. *Escherichia coli* especially and *Aerobacter aerogenes* less frequently were consistently isolated from the skin, mouth, intestines and cloaca. All cultural techniques were standard for the facultative aerobes. No attempt was made to identify fungi, Mycobacteria, sporulating anaerobes or animal parasites, although in a few instances non-sporulating anaerobes were isolated and identified generically only, e.g. *Bacteroides sp.*

Bacteria-free mammals have long been brought into prominence in the study of disease states and antigen/antibody interactions. Amphibians are just beginning to be subjects of interest in bacteria-free research because of the need to control the homogeneity of the stock to ensure the reliability of research data.

The frog is highly useful in animal experimentation, but before its status in experiments requiring homogeneity can be defined, it is necessary to ascertain the organisms that it may characteristically harbor without symptomatology. Much is known about its animal parasites, but very little about its bacterial populations, and it is reasonable to wonder whether bacteria are carried internally in symptom-free frogs. In this study the bacterial flora of grossly healthy frogs obtained from geographically diverse commercial sources were examined.

MATERIALS AND METHODS

Frogs were obtained from several supply houses in the North-Central and Eastern United States. They were inspected for superficial lesions and also for normal color, posture, reactivity and condition of the eyes as gross indices of health.

The frogs were kept individually in water at 4°C in repeatedly cleaned glass containers. These were kept in different rooms since the habitat may contribute to the species of bacteria isolated. The water used was considered to be bacteria-free, based upon bacterial checks performed here by standard techniques.

The animals were isolated for one week to observe signs of any latent disease, following which only grossly healthy frogs were selected for further tests. A total of 125 animals were selected without reference to sex or time of year.

Cultures were made of the skin, cloaca, mouth and nasopharynx. Then the abdominal skin of the chloroformed frog was swabbed with 2% Amphyl (Lehn and Fink) and an incision was made aseptically to sample the peritoneum, blood, spleen, liver, kidney, stomach and colon.

All specimens were placed in thioglycollate, and after 24 hours sub-cultured on blood agar, EMB agar, and SS agar. After another 24 hours, colonies were picked from EMB and SS agars and placed on Difco triple sugar iron agar (TSI); tryptose lactose iron agar (TLI); Simmons citrate agar; Christensen's urea agar; methyl red/Voges-Proskauer broth; and 1% tryptone for indole production. Colonies from the blood agar were picked and direct smears prepared, stained with Gram-stain and identified by morphologic characteristics and zones of hemolysis on the blood agar. Whenever *Staphylococcus* was identified, a coagulase test was performed to ascertain if the or-

ganisms were coagulase positive or negative. In every instance only coagulase negative forms were found.

Within 24 hours after the colonies had been placed on the differential media the data from the reactions were tabulated and the morphologic characteristics of the colony reexamined. Where positive identification could be made, the tests were concluded; otherwise, they were continued for another 72 hours, the biochemical reactions being noted daily. If at the end of 72 hours there was still some doubt, another 24 hours of incubation were instituted and the more definitive testing procedures of typing and/or agglutination were performed. Bacterial contamination was considered to exist in the host if bacteria could be consistently recovered from the same sampling sites.

Results

Bacteria were found to be common inhabitants of the skin and the alimentary canal. The species involved and their frequency of occurrence are listed in Table 1. *Escherichia coli* and *Aerobacter aerogenes* were the most frequent isolates.

No bacteria could be isolated from the blood, peritoneum, liver, lungs, and kidneys even after 96 hours of incubation in the thioglycollate gelatin media. Gross visual necropsy examination revealed no evidence of infection in any of these same bodily structures.

Table 1.—Bacterial Population of Grossly Healthy Frogs (*Rana pipiens*).

Number of Frogs Tested	Commercial Source	Bacteria Isolated	Site, Organ or Tissue Cultured and Number of Frogs Producing the Isolate[1]				
			Skin	Mouth	Stomach	Intestine	Cloaca
40	North-Central (Wisconsin)	Escherichia coli	40	40	None	40	40
		Aerobacter aerogenes	32	34	None	36	35
		Alpha-hemolytic Streptococcus	37	None	None	None	None
		Hem. Coag. (neg.) Staphylococcus	38	None	None	35	38
		Proteus mirabilis	5	5	5	5	5
		Bacteroides sp	None	None	4	None	None
45	North-Central (Illinois)	Escherichia coli	40	42	None	42	42
		Aerobacter aerogenes	36	35	4	30	31
		Non-hem. Coag. (neg.) Staphylococcus	6	26	19	15	None
		Non-hem. Streptococcus	15	None	None	12	1
		Proteus mirabilis	10	None	None	8	8
		Streptococcus fecalis	None	None	None	5	None
20	North-Central (Illinois)	Escherichia coli	19	20	None	18	19
		Non-hemolytic Streptococcus	7	None	None	None	3
		Alpha-hem. Streptococcus	8	None	None	None	None
20	East (South Carolina)	Escherichia coli	20	15	None	20	20
		Aerobacter aerogenes	18	18	3	18	18
		Alpha-hem. Streptococcus	6	10	None	None	None
		Hem. Coag. (neg.) Staphylococcus	5	None	1	None	None
		Proteus mirabilis	8	None	None	8	7
		Pseudomonas aeruginosa	18	18	None	15	15

[1] No bacteria were ever isolated from the spleen, liver, kidney, peritoneum, or blood, so that no data from these areas are presented.

Discussion

It is apparent that bacteria-free frogs cannot be expected from commercial sources, but it also appears that if vigorous frogs are selected by the experimenter after a period of their isolation, they may be expected to remain free of disease. It is cautioned that there may be no firm relation between the locale of the vendor and the geographic source of the frogs. The animals tested here could have come from any region of the country.

It is of interest that bacterial sterility apparently exists in the blood and visceral organs of the grossly healthy frog and that such animals have bacteria only in the expected localities of the skin and alimentary tract, where they appear to reside as commensals. Several species of bacterial pathogens, described by Breed et al (1957), can potentially exist in the blood or tissues of frogs. These organisms, which include aeromonads, pseudomonads, *Brucella*, spirochetes, tubercle bacilli and others have been considered by Kaplan (1953), Reichenbach-Klinke and Elkan (1965), Gibbs et al, (1966), and many other investigators. In all cases where pathogenic bacteria have been isolated, however, there are associated characteristic signs of disease.

Due to the fact that frogs live in water into which they excrete their body wastes, freedom from bacteria has not been considered a practicable goal in the laboratory. A pathogen-free state, however, is readily attainable through careful control of the habitat, even though the skin, mouth, alimentary canal, and cloaca will always contain a normally harmless bacterial flora.

The evidence that reactive, healthy appearing frogs have bacteria-free blood and tissues except for areas exposed to the external environment supplements similar evidence obtained from dogs by Lutsky and Farmer (1966), although it is contrary to the questions raised by Flynn et al (1965) about the pathogen status of commercially produced mice.

The authors are aware of the restrictiveness of the techniques used. Thioglycollate broth, a single medium, might not be productive for all types of organisms that could be present.

No conclusions can be drawn from this study about the relationship between the presence of viruses and gross health. A widespread distribution of Western equine encephalitis virus was demonstrated in symptom-free frogs (*R. pipiens*) in Saskatchewan (Burton et al. 1966).

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Manuscript received Dec. 12, 1967.

FIRST RECORDS OF THE LEAST WEASEL IN MCLEAN COUNTY

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ABSTRACT.—Four records of the least weasel (*Mustela rixosa*) are reported from McLean County, Illinois.

The authors have collected three specimens and have found one mummy of the least weasel (*Mustela rixosa*) near Normal, McLean County. Live-trapping was conducted in a 15 month period and totaled 6,299 trap nights.

On March 28, 1967, a male (testes - 8mm. in length) was trapped along a railroad right-of-way two miles north of Normal. The vegetation consisted mainly of bluegrass (*Poa pratensis*) and miscellaneous weeds. The pelage of this specimen was white below a line from the ears to the base of the tail and brown with a few scattered white hairs on the dorsal surface.

The second specimen, a female with six embryos and six corpora lutea, was trapped May 26, 1967 in a grass waterway one-half mile west of the first capture. The pelage was unprime dorsad and showed the typical summer coat, except that on the feet only the digits were white.

Another female was trapped in a waterway on July 17, 1967 two and one-half miles east of the first capture. The uterus was not enlarged and had no placental scars. The entire pelage was dark brown except for a white throat and chin and a few white hairs in the inguinal region.

The mummified specimen was found September 14, 1967 in the same waterway as the second specimen. It possessed the typical summer pelage.

The first three specimens are preserved as study skins and skulls in the Illinois State University mammal collection (No. 377, 378, 379).

These records provide a midpoint of occurrence for this species between Woodford (Parmalee and Munyer, 1966) and Champaign Counties (Hoffmeister, 1956; Hoffmeister and Mohr, 1957) in central Illinois and indicate that populations are locally extant in this area.

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Manuscript received Oct. 20, 1967.

NOTES ON A NEW METHOD OF COLONY FORMATION EMPLOYED BY POLYERGUS LUCIDUS LUCIDUS MAYR (HYMENOPTERA: FORMICIDAE

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ABSTRACT.—*Polyergus lucidus lucidus* Mayr founds new colonies by budding as opposed to adoption, which is recognized as the method employed by the genus *Polyergus*.

The obligatory slave making ants of the genus *Polyergus* are found in Europe and North America. This genus is considered a classic by myrmecologists because of their beauty and the efficiency with which they conduct raids. Extensive biological studies of the European *Polyergus rufescens rufescens* Latreille have been completed making its habits well known. However, the American *Polyergus*, *rufescens* ssp. and *lucidus* ssp., have not been extensively studied. Most observers are content to assign the habits of the European *rufescens* to the American forms.

There are several short works on the colony formation of the American *Polyergus*, but few are comprehensive. Wheeler (1910, p. 471-487) described in detail the habits of the known species and questioned the assumption that *lucidus* founds colonies by adoption. Creighton (1950, p. 552-560) supplied the most recent taxonomic revision of the genus and described a *lucidus* raid. He stated that adoption is the method employed by *Polyergus* females to found colonies.

The purpose of this paper is to present budding as a method employed by *Polyergus lucidus lucidus* Mayr in colony founding. This discovery is significant because budding is reported only in the subfamilies Dorylinae, Myrmicinae, and Dolichoderinae. This is assumed to be the first instance documented in Formicinae.

MATERIALS AND METHODS. — Previous reports on *lucidus* and most other American *Polyergus* are based on short term studies and chance observations, which often caused conclusions to be drawn from over-extended data. The data used for this report are based on observations made by myself over a four summer period. Ten *lucidus* nests, five located on the same lot in Washington, Ill., were observed. Seven other colonies, five newly founded by budding, did not survive long enough for effective observation.

Direct observation, often for four or five hour stretches, was used in order to secure accurate raiding records. Nests were marked and their position plotted on graph paper. Lines originating on a dead stump on the lot served as the horizontal and vertical axes. By this method I was able to keep yearly records of colony locations.

Identifications were made by G. L. Rotramel, University of California (formerly with the Illinois Natural History Survey); and D. R. Smith, U. S. Department of Agriculture, U. S. National Museum, Washington, D. C. I am indebted to these men for their assistance and encouragement.

RESULTS

Dealate *lucidus* queens return to a *lucidus* nest after mating. They mill about the mound and cling to grass blades. Frequently eight or more dealates are perched around a formicary after a mating flight. Some wander off into the grass, others dart into the entrance, while others attempt to caress slaves and *lucidus* workers which are usually hostile. It appears that the dealates from one nest sometimes seek out other nests, since dealates were found near a nest that had had no mating flight.

Budding takes place when a fertile queen leaves a mother colony with a troop of workers and begins a new colony. A *lucidus* queen and her workers must capture an established *nitidiventris* formicary in order to obtain ma-

ture slaves. I observed three types of budding.

I first observed budding on 28 July 1964, when a column of *lucidus* with a large number of young, orange workers attacked a *nitidiventris* colony. The raid proceeded normally and the attackers began to leave with captured larvae and pupae. Three dealate *lucidus* queens then approached the nest and began milling among the remaining *lucidus* workers. Most of the young workers and some of the older attackers then perched on grass blades near the entrance. Most of the attackers returned in the usual manner. A number of older *lucidus* remained inside the nest and threw uncooperative workers out. Many of the *nitidiventris* were maimed or killed. The other *lucidus* workers and queens milled outside the nest and entered a few at a time. Three days later only a few young *lucidus* remained outside, and these were carried inside by conquered *nitidiventris* workers. This new colony attempted its first raid four days after being established and completed a raid on the fifth.

The second type of budding nearly paralleled the first. Some *lucidus* workers and a dealate milled about a raided nest. But no attackers remained inside the nest after the main force left with the larvae and pupae. Some of the *nitidiventris* workers approached the milling *lucidus* and carried them into an abandoned spider burrow two yards from the nest entrance. The *nitidiventris* also carried many of their own exhausted comrades and rescued larvae and pupae into the burrow. A possible explanation for the unusual behavior of the *nitidiventris* is that immediately after the main body of *lucidus* left, about 100 *Myrmica sabuleti americana* Weber workers attacked the *nitidiventris* workers that were trying to regroup. This formicary was raided again by the same *lucidus* colony two days later.

The most purposeful type of budding was the third, which always occurred late in the raiding season. A single queen was accompanied by about 30 workers on these attacks. The queen spent much time milling about the mound and among the workers before the attack began. A column slowly formed and proceeded toward the victim nest. During the march the queen continually milled among the attackers and was frequently at the column's head. The entire phalanx swarmed into a *nitidiventris* formicary and were met with unusual resistance. The *nitidiventris* were soon defeated. The queen and most of the attackers remained inside the nest.

Queens accompanying columns do not always receive a troop of workers. If a queen cannot induce some attackers to remain at a raided nest, she enters it alone, wanders off, or returns to the *lucidus* nest with the workers.

One evening a *lucidus* colony attacked the same formicary four times. Several *lucidus* dealates appeared and milled about the nest entrance. Some entered. All *lucidus* workers left the area. Some *nitidiventris* workers began carrying their comrades into a small hole two feet from the nest entrance. A *nitidiventris* approached this hole carrying one of the *lucidus* queens. I separated them, and the worker began rubbing antennae with the queen. The worker grasped the queen by the mandible; she assumed pupal posture and was carried into the hole.

I observed lone *lucidus* queens wandering in large arcs over fields. One alate milled about the entrance to a *nitidiventris* formicary for over an hour and entered frequently. Another time a dealate wandered aimlessly for 50 yds. before I lost track of her.

Discussion

Other authors generally agree that *Polyergus* founds colonies by adoption. This is known to be true for *rufescens* ssp. Creighton (1950, p. 555) described the adoption process. A lone *rufescens* queen enters a slave nest, gradually becomes accepted, kills the queen mother, and is then cared for by the workers. The queens meet considerable opposition when introduced into artificial *Formica fusca* nests and must often kill defenders. Wheeler (1916, p. 108) found a *rufescens breviceps* queen in an incipient *fusca* colony. I made a similar observation.

Wheeler (1910, p. 486) conducted a series of experiments with *lucidus* queens which caused him to leave the question of *lucidus* colony formation unanswered in his book. In 1964 I conducted similar experiments in three jars containing *nitidiventris* workers and pupae from a raided nest. A dealate *lucidus* queen was placed inside each jar. Immediately the queens approached the workers and stroked them with their antennae. Workers killed the first

queen. In the second jar the queen killed the workers. And in the third, the queen and workers huddled together peacefully. Though most attempts at artificially causing *lucidus* queens to seek adoption are negative, field observations indicate that *lucidus* either does or did use this method. But the instances of *lucidus* queens being carried off by harrassed *nitidiventris* workers are much more passive than the standard *rufescens* method of adoption. Queens which enter *nitidiventris* nests alone may actually become adopted, or they may end up like those in the jars. More field and laboratory observations are needed before the questions connected with *lucidus* adoption can be answered.

Wheeler (1916, p. 110 & 114) saw dealate *rufescens breviceps* queens accompany workers on raids and then return to their formicaries. He also stated that queens of the European *rufescens* sometimes accompany attackers. He suggested that these queens either seek immediate adoption into the raided nest while the defenders are still disorganized, or that they are scouting area slave nests.

The different forms of *Polyergus* evolved closely. Smith (1947), Creighton (1950), and Forbes and Brassel (1962) all reported the difficulties connected with the taxonomic identification of the various forms. The biology of the different forms is also nearly parallel. The main difference between the raids of *lucidus* and *rufescens* is that *lucidus* is more precise, while *rufescens* uses much larger armies. The colony founding activities of the queens is another indication of close evolution.

The fact that *rufescens* queens follow raiding columns is an indication that this species may bud. This is unlikely since *rufescens* has been well studied. The queens probably follow the columns in obedience to an instinct that is no longer prominent in *rufescen's* colony founding activities. Similarly *lucidus'* strong inclination toward adoption indicates an evolution in its colony founding activities. More field work will eventually bring more evidence to light.

Budding occurs early in June and during the mating season (mid-July—early Sept.). The results of budding expeditions are difficult to verify since the new colonies have a tendency to be inactive for several weeks after establishment and sometimes relocate. Of seven budding attempts, I am sure that one succeeded and one failed. The progress of the others could not be followed. However, in a 1500 sq. ft. area where there were three witnessed attempts between 1964 and 1965, three new *lucidus* colonies appeared in 1966. These formicaries were all at least ten feet from the original bud-nests. While this observation does not prove the success of budding, it does show that *lucidus* colonies are prolific.

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Manuscript received August 26, 1967.

METHODS OF CULTIVATION, ISOLATION, AND INOCULATION OF MYCOPHAGOUS NEMATODES INTO NEMATOPHAGOUS FUNGUS CULTURES

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ABSTRACT.—Instructions for the cultivation, isolation, and inoculation of mycophagous nematodes are presented. The methods utilized allowed large numbers of fungus-feeding nematodes to be reared and inoculated into nematode-trapping fungus cultures.

Aphelenchus avenae Bastian, and *Neotylenchus linfordi* Hechler, are two fungus-feeding nematode species that were used in a study of the biological relationships between nematophagous fungi and mycophagous nematodes (Monoson, 1967). Both species of nematodes have been used previously in reproductive and feeding evaluations with non-predaceous fungi (Hechler, 1962a; 1962b; Pillai, 1966).

A constant supply of *Aphelenchus avenae* and *Neotylenchus linfordi* were necessary for the investigation on the activities of five predaceous fungi that actively capture and kill living nematodes. The present paper provides information concerning the methods used in the cultivation, isolation, and inoculation of *A. avenae* and *N. linfordi* into nematophagous fungi cultures.

Cultures of *Aphelenchus avenae* and *Neotylenchus linfordi* were maintained on one-quarter-strength Difco potato-dextrose agar at room temperature. The mycophagous nematode species and their non-predaceous food source (*Pyrenochaeta terrestris* (Hansen) Gorenz, J. C. Walker, and Larsen) were transferred together every week to new one-quarter-strength Difco potato-dextrose—agar medium. Two-week-old petri-dish cultures of nematodes were scanned using a binocular microscope and areas of nematode concentrations were marked off with a marking crayon on the glass surface of the dish. From these nematode-concentration areas wedge-shaped pieces of agar were cut out and the inoculum was transferred to the new medium. The method allowed for the maintenance of nematodes in all stages of development as well as high physiological state of feeding and reproduction. All nematode transfers were made by using a positive pressure inoculation chamber in order to exclude any possible contamination of the cultures.

For the purpose of this study nematodes were grown for two weeks and then harvested for inoculation with nematophagous fungi. The contents of one petri dish usually provided sufficient nematode numbers for most inoculations.

A simple extraction apparatus was formed by placing four layers of Kimwipe tissues between two plastic funnel tops which rested in a Syracuse dish (Edwards, 1962). This apparatus and most glassware used were steam sterilized at 18 psi for 15 minutes. Nematodes were separated from the agar medium by pouring approximately 10-15 ml of sterile distilled water over the surface of nematode stock cultures. Nematodes readily moved into the surface water and were unable to reenter the agar substrate. *Pyrenochaeta terrestris* produced negligible amounts of conidia in culture and the water suspension was, therefore, essentially devoid of fungus material. The water suspension was then transferred to the extraction device by pouring the water over the Kimwipes. The apparatus was placed over the Syracuse dish and the water was allowed to filter through the Kimwipes. After one-half hour several thousand nematodes had moved down into the Syracuse dish. The process was repeated several times in order to harvest the greatest number of nematodes which were present. Nematodes collected in this manner were clean but were further washed three times in sterile distilled water by centrifugation. Nematodes cleaned by this simple method did not exhibit any bacterial or

fungal contamination when transferred to cultures containing nematophagous fungi.

Extracted nematodes were placed in a sterile measuring dispenser (distributed by Chemical Rubber Company, Cleveland, Ohio) with approximately 45 ml of sterile distilled water. The glass dispenser and measuring head had been sterilized in 70 per cent alcohol for 15 minutes and then had been thoroughly rinsed with sterile distilled water. This method eliminated the possibility of inoculation variations which may have resulted from repeated steam sterilization of the dispenser apparatus.

One-milliliter aliquots of the nematode suspension were taken and the nematodes were counted in a plastic counting dish. Subsequent 50 ml dilutions with sterile distilled water resulted in a final population of approximately 400 nematodes per ml of liquid. This final population was used either as an inoculation amount or as a base population from which all other dilutions were made.

Inoculations of nematodes into nematophagous fungus cultures were made either directly from the dispenser apparatus or from serially diluted solutions with the aid of a calibrated 1 ml duplicating pipette (distributed by E. H. Sargent and Company, Chicago, Illinois). Prior to each inoculation the liquid was swirled so that each withdrawal of liquid contained a homogeneous sample of the inoculum. One milliliter of liquid was the amount inoculated into petri dishes containing nematophagous fungi. The plates were left uncovered in a positive pressure inoculation chamber for 15 minutes so as to allow the 1 ml of liquid to evaporate. After 15 minutes sterile petri-dish lids were carefully placed over the bottom sections.

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Manuscript received Sept. 22, 1967.

FIELD NITROGEN FIXED BY NODULE BACTERIA AND SOME SYMBIOTIC RELATIONSHIPS EXHIBITED BY TWO SOYBEAN SELECTIONS

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ABSTRACT.—Two sister selections of soybeans under varying soil nitrogen levels fixed amounts of nitrogen comparable to values reported by other workers using other measurement techniques. Preliminary observations are reported on differences shown by the symbionts under laboratory conditions.

A number of methods have been used to determine the amount of nitrogen fixed by legumes in the symbiotic process under field conditions. Many early workers compared the nitrogen levels of nodulated and non-nodulated legumes as a criterion of fixation. (Virtanen and Linkola, 1947) compared the nitrogen content of plants nodulated with effective strains with those nodulated with ineffective strains. Using peas they found that under field conditions, more than 88 lbs. N/A was fixed annually. The generalization most widely used to describe the contribution of nodule bacteria to the leguminous crop has been that of (Hopkins, 1910). It is generally stated that ⅓ of the nitrogen in the crop comes from the soil and the remaining ⅔ from the fixation of atmospheric nitrogen.

Other workers have attacked this problem using the N_{15} isotope to measure the nitrogen made available to the plant through the fixation process when supplied with varying amounts of soil or fertilizer nitrogen. (Norman and Krampitz, 1946) using the N_{15} technique with soybeans reported that the fixation process at any level of supplied nitrogen was not completely halted nor was all of the inorganic nitrogen recovered in the crop. The presence of fertilizer nitrogen largely resulted in a dimunition of the amount of atmospheric nitrogen fixed in the symbiotic process. (Thornton, 1947) using tracer techniques with inoculated lespedeza and soybean plants found a reverse relationship between the fixation of atmospheric nitrogen and the amount of inorganic nitrogen made available to the plant. From these experiments an estimate was made that in prairie soils of average nitrogen content not more than 30% of the nitrogen in the plant is air derived.

A more extensive experiment utilizing 7 leguminous and 4 non-leguminous plants was conducted by (Allos and Bartholomew, 1955). Nitrogen was added as ammonium sulfate and was enriched with the N_{15} isotope to the extent of 1.65 atom percent. Nitrogen fixation decreased while fertilizer nitrogen uptake increased as the quantity of available nitrogen to the plants was increased. As in previous observations no total inhibition of fixation or total absorption of the fertilizer nitrogen was achieved. Nitrogen fixation was largest in the soybean and peanut plants which were also the plants with the greatest total growth. In the case of the soybean plant they state that "When nitrogen was applied in quantities equal to or in excess of that absorbed by the soybean crop, fixation from the atmosphere did not constitute more than 30 to 40% of the total absorbed. When nitrogen was applied equal to 10 to 15% of that absorbed by the crop, fixation contributed from 80 to 90% of the total absorbed."

Williams and Lynch (1954) reported in 1953 two strains of soybeans that varied only in their ability to form nodules. One of these is abundantly nodulated, while the other strain bears no nodules under field conditions. Genetic studies indicate that the inability to nodulate was due to a single recessive gene. Sears and Lynch (1951) have made some preliminary reports on these varieties when studied in the field. It was found that given adequate sources of nitrogen and other nutrients, the two sister strains gave comparable yields of soybeans in the field. Some

of these data have been reported elsewhere (Sears and Lynch, 1951) and are given here for comparison purposes with other techniques used for the measurement of nitrogen fixed by nodule bacteria under field conditions.

Methods and Materials

The two sister strains varying only in the ability to produce nodules were used to determine the contribution of nodule bacteria to the soybean crop and to determine what percentage of the nitrogen in nodulated soybeans is obtained from the air. The soybean strain that bears no nodules is designated as the low protein selection, while the nodulated strain is designated as the high protein selection.

The two sister strains were inoculated with the same strain of nodule bacteria and seeded in plots that differed only in their nitrogen content. Other nutrients were supplied in adequate amounts. The soil treatments used are given in Table I.

Treatment I was used to demonstrate that the two soybean selections had equal yielding abilities. The straw in treatment V was used to reduce the soil nitrogen supply available to the soybean plants. Some of the beans and plant parts were harvested for chemical analyses while others were harvested for yield comparison purposes.

Laboratory studies of the nutritional requirements of these microorganisms were carried out on various media to which vitamins or casein were added. Cultural characteristics such as pigment production, rate of growth, slime production, and colonial characteristics were recorded.

Results and Discussion

The seed yields and nitrogen contents of the entire plants, including roots are given in Table 2. It can be seen that the greatest benefit from the nodule bacteria occurs on soils that have low nitrogen levels. In fact the use of nitrogen fertilizers actually decreases the nitrogen available from nodulation. It also suggests that the soybean plant can attain maximum yields by nodulation alone if other nutrients are not limiting. These results are in conformity with those of (Thornton, 1947) and (Allos and Bartholomew, 1955) who report an inverse relationship between the amount of nitrogen available to the plant and the amount fixed in the symbiotic process. If one uses the estimate that 1.5 pounds of soil nitrogen is sufficient to produce a bushel of corn and if the corn plant can obtain amounts of nitrogen from the soil equivalent to that obtained by the soybean plant then the following conclusions can be reached. On land having a nitrogen fertility level sufficient to produce a 40 bushel corn crop per acre nearly two-thirds of the soybean nitrogen requirement will be secured from the air. On land of 100 bushel per acre status, only about one-fifth of the nitrogen in the nodulated soybean crop will be from the atmosphere. These results also coincide with the results reported by (Allos and Bartholomew, 1955) in that the soybean plant gave little or no growth response to nitrogen fertilizer treatments.

It should be pointed out that under field conditions the low protein selection was never observed to be nodulated. However, in sand and vermiculite a few inefficient nodules were found on the low protein selection when grown in the

Table 1.—Soil Treatment Practices.

Treatment	Previous Treatment	Additional Treatment
I.....	Birdsfoot trefoil green manure crop.....	NH_4NO_3 300 lb. per acre
II.....	Same as Treatment I.....	none
III.....	Grass sod fertilized with $(NH_4)_2SO_4$.....	none
IV.....	Grass sod—no nitrogen fertilizer.....	none
V.....	Same as Treatment IV.....	5-ton oat straw mulch

TABLE 2.—Yields and Nitrogen Content of Soybeans on Soils of Different Nitrogen Fertility Levels.

Treatment	Soybean Selection		Total N		N from Air
	High Protein	Low Protein	High Protein	Low Protein	
	bu./A	bu./A	lbs./A	lbs./A	%
I	38.6	38.3	.....	.....	.....
II	36.2	33.0	.....	.....	.....
III	36.6	33.1	199	158	21
IV	36.0	29.2	192	102	47
V	35.5	20.9	177	58	63

greenhouse. A study of those few strains of soybean nodule bacteria that would effect some inefficient nodulation, as against the many strains that would not, yielded some general differences in cultural and biochemical characteristics of these *Rhizobia*. The few nodulating strains produced a thin slimy growth with a distinct pigment, they grew at a faster rate, elaborated indole-3-acetic acid from casein and had fewer vitamin requirements (especially B vitamins) than did the non-infective strains. These differences and others are now being studied to ascertain the physiological bases for the nodulation of leguminous plants.

This experiment was unique in that two sister strains of soybeans varying in nodulating ability were used to determine the contribution made by nodule bacteria to a crop under field conditions and varying levels of soil nitrogen. With very inadequate supplies of soil nitrogen as much as 63% of the nitrogen in the crop comes from the air by bacterial fixation. Under high nitrogen levels only about 21% is so obtained (Sears and Lynch, 1951).

In this era of increasing populations the use of efficient nodule bacteria in many areas of the world should be of considerable economic importance.

ACKNOWLEDGMENT

A part of this work was done in conjunction with O. H. Sears, Dep't. of Agronomy, University of Illinois.

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Manuscript received July 3, 1967.

CALCULATED FUNDAMENTAL VIBRATIONAL FREQUENCIES OF ISOTOPICALLY SUBSTITUTED OZONE

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ABSTRACT. — The fundamental vibrational frequencies of eighteen distinct isotopically substituted ozones have been calculated.

Oxygen consists of three stable isotopes, oxygen—16, oxygen—17, and oxygen—18. As a result there exists the possibility of forming eighteen distinct isotopically substituted species of ozone. Nine species belong to the group C_{2v}. These are the bent X_3 or XY_2 molecules. The remaining nine species, the bent XXY or the bent XYZ molecules, belong to the lower symmetry group C_s.

Wilson's FG matrix method (Wilson, Decius, Cross, 1955) was used to calculate the vibrational frequencies for the isotopically substituted ozones. Explicit expressions for the F's and the G's used for the molecules belonging to the group C_{2v} are given by Nakamoto (1963; p. 264). Expressions for the F's and the G's used for the molecules belonging to the group C_s are given by Polo and Wilson (1954).

The structural parameters of ozone and the values of the potential constants used in the F matrix were taken as those determined by Pierce (1956). These values, as well as the values of the atomic masses used in the calculations, are listed in Table I.

The calculations of the G's, F's, and subsequent multiplications and solutions of the resultant secular equations were carried out on an IBM 1620 electronic computer. Twelve significant digits were carried in all calculations. The results of the calculations are listed in Table II.

DISCUSSION

The calculated frequencies in cm^{-1} agree well with those reported by Pierce (1956).

$^{16}0_3$ observed: 1110, 705, 1043; calculated: 1110.00, 704.99, 1042.99; $^{16}0$ $^{18}0$ $^{16}0$ reported by Pierce (1956): 1080, 697, 1008; calculated this work: 1079.91, 697.22, 1008.00; $^{16}0$ $^{16}0$ $^{18}0$ reported by Pierce (1956): 1095, 688, 1029; calculated this work: 1096.82, 688.23, 1028.45. The minor discrepancy observed in the last case represents less than 0.2% deviation.

A check on the accuracy of the vibrational frequency calculations is afforded by an application of the Sum Rule, (Wilson, Decius, Cross, 1955; p. 186) which states that for the case of ozone:

$$\sigma(^{16}0_3) + \sigma(^{18}0_3) - \sigma(^{16}0\ ^{16}0\ ^{18}0) - \sigma(^{16}0\ ^{18}0\ ^{18}0) = 0$$

and

$$\sigma(^{16}0_3) + \sigma(^{18}0_3) - \sigma(^{16}0\ ^{18}0\ ^{16}0) - \sigma(^{18}0\ ^{16}0\ ^{18}0) = 0$$

where σ represents the sum of the vibrational frequencies for the indicated species.

For the cases listed above agreement is about 0.2%, which is considered good since the Sum Rule takes no account of electrical anharmonicity and vibrational-rotational interactions.

TABLE I

Structural parameters and potential constants of ozone.

$0 - 0 - 0 = 116° 58'$
$0 - 0$ bond length $= d_o = 1.276$ Å
$^{16}0$ mass $= 16$ amu
$^{17}0$ mass $= 17.004533$ amu
$^{18}0$ mass $= 18.00487$ amu
$f_d = 5.7007 \times 10^5$ dyne cm^{-1}
$f_{dd} = 1.5233 \times 10^5$ dyne cm^{-1}
$f/d_o^2 = 1.2847 \times 10^5$ dyne cm^{-1}
$f_d/d_o = 0.3324 \times 10^5$ dyne cm^{-1}

TABLE II

Vibrational frequencies of isotopically substituted ozone.

SPECIES	$\nu_1(cm^{-1})$	$\nu_2(cm^{-1})$	$\nu_3(cm^{-1})$
$^{16}0_3$	1110.00	704.99	1042.99
$^{17}0_3$	1076.72	683.85	1011.72
$^{18}0_3$	1046.38	664.58	983.21
$^{17}0\ ^{16}0\ ^{17}0$	1093.18	687.50	1030.36
$^{18}0\ ^{16}0\ ^{18}0$	1078.29	671.31	1019.05
$^{16}0\ ^{17}0\ ^{16}0$	1094.02	701.06	1024.58
$^{18}0\ ^{17}0\ ^{18}0$	1061.37	667.92	1000.20
$^{16}0\ ^{18}0\ ^{16}0$	1079.91	697.22	1008.00
$^{17}0\ ^{18}0\ ^{17}0$	1062.14	680.28	994.93
$^{17}0\ ^{16}0\ ^{18}0$	1086.37	679.42	1024.10
$^{16}0\ ^{17}0\ ^{18}0$	1080.42	684.57	1009.76
$^{16}0\ ^{18}0\ ^{17}0$	1071.80	688.77	1000.72
$^{16}0\ ^{16}0\ ^{17}0$	1102.34	696.27	1035.95
$^{16}0\ ^{16}0\ ^{18}0$	1096.82	688.23	1028.45
$^{16}0\ ^{17}0\ ^{17}0$	1086.13	692.48	1017.41
$^{17}0\ ^{17}0\ ^{18}0$	1069.69	675.90	1005.34
$^{16}0\ ^{18}0\ ^{18}0$	1065.91	680.98	992.94
$^{17}0\ ^{18}0\ ^{18}0$	1054.91	672.45	988.44

The low isotopic abundance of oxygen-17 and oxygen-18 probably precludes observations of infrared data on these isotopically substituted ozone species. Infrared data on oxygen-16 and oxygen-18 ozone species could be obtained from oxygen-18 enriched ozone.

ACKNOWLEDGEMENT

The assistance of Mr. Charles Fincke of the Data Processing and Computing Center, Southern Illinois University, Edwardsville, is gratefully acknowledged.

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Manuscript received July 23, 1967.

COMPUTER CALCULATION OF INFORMATION — THEORETIC MEASURES OF DIVERSITY

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ABSTRACT. — Computational methods are suggested which facilitate implementation of computer calculations of both the Shannon and Brillouin measures of information content.

The calculation of the information content of a collection of entities has been performed for several sorts of biological considerations (Quastler, 1953), and the use of information content as a measure of ecological diversity has been of special interest in recent years (Margalef, 1957; MacArthur, 1965; Pielou, 1966a, 1966c). An aggregation of items which is high in information content is high in uncertainty, entropy, and diversity, and the information content of ecological communities has also been considered in relation to stability (MacArthur, 1955; Leigh, 1965) and "maturity" (Margalef, 1963).

METHODS

The mean diversity, per individual (i), in a collection of N individuals belonging to s categories (e.g. species) may be calculated by the measure of Shannon (1948):

$$H' = -\sum_{i=1}^{s} p_i \log p_i \qquad (1)$$

or by that of Brillouin (1956):

$$H = \frac{1}{N!} \log \left(\frac{N!}{n_1!\, n_2! \ldots n_s!} \right) \qquad (2)$$

where n_i is the number of individuals in the ith category and p_i is n_i/N, the proportion of N which belong to category i.

These two measures are not identical. The relative merits of each are dependent upon the population sampling represented by the data (Pielou, 1966b, 1966c). Lloyd, Zar, and Karr (1968) have shown that given a set of data, the calculations of H and H' are equally simple if certain manipulations are performed. Thus the type of sampling one's data represent, and not the ease of calculation, should dictate which diversity measure to compute. For ease of calculation, Shannon's measure may be written as:

$$H_b' = \frac{c}{N} \left[N \log_a N - \sum_{i=1}^{s} n_i \log_a n_i \right] \qquad (3)$$

and the Brillouin formula may be written as:

$$H_b = \frac{c}{N} \left[\log_a N! - \sum_{i=1}^{s} \log_a n_i! \right] \qquad (4)$$

so that one needs only a table of $\log n!$ and $n \log n$ to calculate both H and H' with equal ease; such a table is available in base 10 (Lloyd, Zar, and Karr, 1968). The calculations may be performed using logarithms of any base, a, and the resultant H or H' may be expressed in any other base, b, by inserting the appropriate scale factor, c, in formulae (3) and (4). Bases commonly used are e, 2, and 10, where the unit of information is called the "nit", "bit", and "hartley', respectively (Table 1).

For workers performing these information-theoretic calculations routinely, a digital computer program offers great conservation of time and insurance against miscalculations. The efficiency of such computer programs is greatly enhanced by the use of formulae (3) and (4) rather than the parent formulae (1) and (2). Using formula (3) eliminates the need of computing the proportions $p_i = n_i/N$ a total of s times and then summing the s number of $p_i \log p_i$ values. Formula (4) avoids the possibility of overflowing computer capacity when calculating factorials, for addition of logarithms is used for the

TABLE 1.—Scale factors (c) to convert from logarithmic base a to base b.

b	a		
	2	e	10
2.....	.....	1.442695	3.321928
e.....	0.693147	.....	2.302585
10.....	0.301030	0.434294	.....

necessary multiplication of the integers and factorials. The calculation of *log* $n!$ as $\sum_{m=2}^{n}$ *log* m is not without disadvantage, however, even on a computer. Regardless of the accuracy of each calculation of *log* m, there is some finite error associated with it. These errors, however small, are being summed, and generally result in a low approximation of *log* $n!$. Using eight significant figures throughout the calculations, the calculation of *log* $n!$ by the summing of logarithms may accumulate an error in the sixth significant figure well below $n = 50$. The use of "double precision" computer arithmetic serves to delay the appearance of the error, but it may do so at considerable cost in computer storage and time. The use of the logarithmic form of Stirling's formula for approximation of factorials:

$$\log n! = (n+\tfrac{1}{2}) \log n + \tfrac{1}{2} \log 2\pi - n \log e \qquad (5)$$

(Selby, 1965: 340) allows one to estimate the *log* $n!$ value desired in one algebraic computation. While this approximation is good only for large n, the related formula:

$$\log n! = (n+\tfrac{1}{2}) \log n + \tfrac{1}{2} \log 2\pi - \left(n - \frac{1}{12n} + \frac{1}{360n^3}\right) \log e \qquad (6)$$

(Fisher and Yates, 1963: 129) can estimate *log* $n!$, for n as small as 4, accurate to at least the 6th significant figure ($n = 1$ through $n = 2000$ have been tested). If natural logarithms are used, *log* $e = 1$ and the equation is somewhat simplified. It may also be noted that n need not be an integer to be used in this formula.

If the data are a large random sample, the standard error of H':

$$\text{S.E. } (H') = \sqrt{\frac{[\Sigma p_i \log^2 p_i - (H')^2]/N}{}} \qquad (7)$$

(Basharin, 1959) may be calculated readily as:

$$\text{S.E. } (H') = \frac{c}{N} \sqrt{\Sigma n_i \log^2 n_i - (\Sigma n_i \log n_i)^2/2N} \qquad (8)$$

Also of interest is the determination of the maximum diversity possible with the given s and N. This may be calculated as:

$$H_{max}' = \log s \qquad (9)$$

or

$$H_{max} = \frac{c}{N} [\log N! - (s\text{-}r) \log D! - r \log (D+1)!] \qquad (10)$$

where D is the integer part of N/s and $r = N - s \cdot D$.

The evenness of the collection (Pielou, 1966c) is $J = H/H_{max}$ using the Brillouin measure, and $J' = H'/H_{max}'$ using Shannon's formula.

The minimum diversity possible is given as:

$$H_{min} = \frac{c}{N} \left[\log N! - \log Q! \right] \qquad (11)$$

or

$$H_{min}' = \frac{c}{N} \left[N \log N - Q \log Q \right] \qquad (12)$$

where $Q = N - (s - 1)$. The redundancy, R, is:

$$R = \frac{H_{max} - H}{H_{max} - H_{min}} \qquad (13)$$

and of course R' may be computed in an analogous fashion using Shannon type measures throughout. One may also arrive at an index of heterogeneity for the collection as

$$R_H = 1 - R \quad \text{or} \quad R_H' = 1 - R'.$$

A FORTRAN IV computer program has been prepared which calculates H,

H', S.E. (H'), H_{max}, H_{max}', H_{min}, and H_{min}' in bases e, 2, and 10, and also calculates J, J', R, and R'. User's options include the choice of computing the Shannon and/or Brillouin measures. A program listing, with instructions for use, is available on request.

Acknowledgments

These computer procedures were developed using the IBM 360 and IBM 7094/1401 facilities of the University of Illinois (partially supported by National Science Foundation grants, with time granted to the Zoology Department from Public Health funds of the University of Illinois Research Board). Many of the program features were suggested by James R. Karr.

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Manuscript received November 4, 1967.

THE THERMODYNAMIC SOLUBILITY PRODUCT CONSTANT BY CONDUCTIVITY MEASUREMENT

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ABSTRACT.—A procedure for correcting solubility product constants to account for effects of activity and concentration dependence of conductivity is presented. This procedure suggests additional treatment of data in the usual conductivity determination of solubility product constants in the undergraduate physical chemistry laboratory.

A common laboratory experiment in an undergraduate course in physical chemistry is the determination of the solubility of a sparingly soluble salt through the measurement of the specific conductivity of a saturated aqueous solution of the salt. In this determination one uses the equation

$$\Lambda_c = \frac{1000\, L_s}{c} \qquad (1)$$

where L_s is the specific conductivity of the salt in the saturated solution, c is the concentration of the salt in equivalents per liter and Λ_c is the equivalent conductivity of the solution. It is assumed that for dilute solutions, Λ_o, the equivalent conductivity at infinite dilution, may be substituted for Λ_c without introducing any appreciable error. The experimentally determined value of the specific conductivity of the salt in solution and the value of the limiting equivalent conductivity, available in the literature, are introduced into Equation 1 in order to determine the equivalent concentration of the salt and, hence, its solubility product constant.

Using the equations of the Onsager theory of conductivity and the limiting form of the Debye-Hückel Theory, a correction may be applied to the solubility determination outlined above and an estimate made of the thermodynamic solubility product constant.

To illustrate this procedure, the example of lead sulfate will be considered. This salt is a commonly suggested one in laboratory manuals. Because lead sulfate is a 2-2 valent electrolyte, the corrections to be made are quite striking.

A typical student determination yielded a specific conductivity of 4.38×10^{-5} ohm^{-1} cm^{-1} for lead sulfate at 25°C. Inserting this value and a value (Robinson and Stokes, 1965, p. 465) of $\Lambda_o = 149.5$ ohm^{-1} for the concentration of the saturated solution gives a value of $K_{sp} = 2.10 \times 10^{-8}$ mole^2 l^{-2}.

The value of the concentration so derived may be substituted for the concentration term in the Onsager Limiting Law (Glasstone, 1948, p. 904)

$$\Lambda_c = \Lambda_o - (A + B\Lambda_o)\sqrt{c} \qquad (2)$$

where the terms A and B refer to the electrophoretic effect and the asymmetry effect, respectively. These terms are dependent upon temperature, solvent, ionic charges and the limiting conductance of the ions. The student may now calculate a value of Λ_c from Equation 2 and substitute this value for Λ_o in Equation 1 and obtain a new value for the concentration of lead sulfate. This process is repeated until a consistent value is obtained for the concentration. In this example, four cycles were performed and produced a value of $C = 3.04 \times 10^{-4}$ eq l^{-1} and $K_{sp} = 2.32 \times 10^{-8}$ mole^2 l^{-2}.

From the relationship for the thermodynamic solubility product constant (Glasstone, 1948, p. 962)

$$K_a = K_{sp}\, \gamma^2 \qquad (3)$$

and from the Debye-Huckel equation for the mean activity coefficient (Glasstone, 1948, p. 956)

$$\log \gamma = -0.509\, Z_+ Z_- \sqrt{\mu} \qquad (4)$$

where μ is the ionic strength of the solution calculated from the final concentration for lead sulfate, a value of $K_a = 1.84 \times 10^{-8}$ mole^2 l^{-2} can be obtained.

Although this series of calculations does not result in any refinements in the value of K_a with regard to those values reported in the literature, it does illustrate to the student the effects of these corrections and the extent to which the approximation for Λ_c may be adjusted. By comparing these results with a 1-1 valence type electrolyte, further significance is given to the influence of charge in the interionic attraction theory.

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Manuscript received September 7, 1967.

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PREPARATION OF MANUSCRIPTS FOR THE TRANSACTIONS

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Transactions

of the

Illinois State Academy of Science

Volume 61
No. 3
1968

Springfield, Illinois

TRANSACTIONS of the ILLINOIS STATE ACADEMY OF SCIENCE

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(53070—6-68)

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TRANSACTIONS

OF THE

ILLINOIS STATE ACADEMY OF SCIENCE

VOLUME 61 - 1968 No. 3

Illinois State Academy of Science

affiliated with the

Illinois State Museum Division

Springfield, Illinois

PRINTED BY AUTHORITY OF THE STATE OF ILLINOIS

Samuel H. Shapiro, *Governor*

September 1, 1968

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THE IMPACT OF SCIENCE IN A DEMOCRACY

MILTON D. THOMPSON
Illinois State Museum, Springfield

The comments of Dr. Norman D. Levine last year in his presidential address, "The Woes of a Biologist"; my involvement with the Illinois Sesquicentennial Commission; and my responsibilities as Director of the Illinois State Museum: all have combined to make me very aware of the multiplicity of problems involved in the understanding and communications of scientific knowledge.

I realize that, as members of an interdisciplinary organization such as the Academy of Science, you are cognizant of the problems of communication between scientists, that you are more or less involved both in the infinite minutiae of specialization and the consolidating gap-closing effect of interdependency and interrelationship among all the facets of mathematics, physics, chemistry, biology, and social sciences.

Because of the complexity of this oneness of the sciences, most major scientific projects are performed today not by individuals but by teams — sometimes referred to as "big science" — whether they be as spectacular and concentrated as the recent heart transplant operations or of a more common occurrence, such as, an archaeological exploration with a working organization of anthropologists — combining archaeologist, physical anthropologist, ceramics analyst, identification laboratory for faunal remains, identification laboratory for floral remains, and a laboratory for Carbon-14 analysis, to name but the most obvious. Not only is this exhaustive exploration of prehistoric sites becoming infinitely more complex, but in the last thirty years, there has been a complete change from a search for archaeological sites to explore to the realization that there is more information to be obtained in Illinois alone than there is manpower or funds for the work. Unfortunately, the physical development of our State — urban development, highway expansion, strip mining, reservoir impoundments, and industrial plant expansion — will destroy the major portions of this material within the next twenty years. A similar explosion in the search for knowledge and in the need for trained manpower is occurring in every one of your fields.

The march of scientific discovery has been a major factor in changing the way of life for mankind upon this earth. Today the proliferation of new knowledge progresses at an uncomprehensible r a t e . However, the impact of any new disclosure on our existence follows or comes after

a very considerable time lag between the period of discovery and the period of implementation. Seldom does a breakthrough from discovery to mass use move as rapidly as did Dr. Salk's discovery of the polio vaccine. The dramatic impact and outstanding results were triggered, of course, by the great need of both the medical profession and the public, supported by full cooperation of mass media communications. This is an example of the techniques of science applied to the solving of a problem of great public concern, but even this project suffered a great emotional and technological setback when the breakdown of techniques in one company laboratory resulted in the spread of a dangerous polio virus.

Basic scientific research does not mean the search for a product of immediate practical use. More commonly, it is simply the search for knowledge: the statement of a problem, the search of the literature, the finding of answers to the unknown facets of knowledge, evaluating the discoveries and publishing the results. The new knowledge added to the total fabric must then be disseminated.

Just a very few years ago a dedicated scholar could set out, as did Sir Francis Bacon, to make all knowledge his; and, egotistical as the objective may seem, Bacon did study and understand the great mass of known recorded knowledge of his time. Imagine a man today becoming an authority on the entire aspect of the science to which he is dedicated, even with the mass assimilating aids that computerization has put at our disposal.

We, as individuals, are increasingly faced with the growing problem expressed in the cliché of "knowing more and more about less and less until we are in danger of knowing everything about nothing." In reality the converse is true.

As I have come to know many of you as individuals and have sat in meetings where your counsel is sought and given, I have come to have great respect for the breadth of your knowledge and the diversity of your interests. Most of the scientists I have come to know, particularly those of you who carry great responsibility as well as having earned stature in your chosen field of science, have proved to be extremely interesting people with a great variety of interests and social skill. Apparently, the ability to communicate with others is a necessary adjunct to scientific recognition. Certainly it is important to the dissemination of scientific knowledge.

As the director of an important science museum dedicated to the preservation of materials and dissemination of knowledge to the public, standing in that tenuous realm between the exploding mass of knowledge and the layman's comprehension (and each of us is a layman when we move out of our particular specialty), I am both challenged and appalled by the problems of interpretation which face us.

In the difficult field of biological interpretation, the task does not seem as great to me as in that of archaeology, probably because the biological sciences have a broader base of generally understood knowledge which has been more stable over a longer period of time; yet much of

what was taught to us a generation ago as fundamental knowledge we now know is "not necessarily so." When we move into a field such as American archaeology, we find that the whole field of knowledge is in such a tremendous state of flux that even our own staff working on the same or closely related projects may have differences of opinion in interpretation — so basic that our interpretive program of exhibits is least developed in the field where we are most deeply committed in essential research.

The exhilaration in the accumulation of knowledge is but a part of the over-stimulation of our senses. Just think how the merchandising fields compete for our every attention and how often they use a clever sort of pseudoscience to rake in our dollars; how often an actor in lab coat with some staged, scientific background of instruments, equipment, or library gives a pronouncement on the special qualities of a medicinal treatment or gasoline ingredient. They recognize the respected position of science but tend to break down its image by trading on its reputation of integrity until the source of the image becomes threatened.

It behooves those of us working in the fields of both basic research and applied science to be doubly careful of our responsibilities. Lest we too become pseudoauthoritarian, we should be most careful of our pronouncements when we move out of our own fields of knowledge into those areas where our emotions, beliefs, and statements are those of a layman and not of a practicing scientist. Here we must so carefully avoid using our positions of prestige and our claims of academic freedom to make authoritative statements which may be destructive and prejudiced. We have so much to defend in areas where we know our ground that we must not weaken our position by injudicious pronouncements and crusades where our prejudices are showing.

The lack of understanding between scientists is not as serious as is the problem of the scientists' communications with those who are completely outside the realm of science. This dilemma creates a major problem and responsibility, especially in a democracy. The acceptance of new concepts by the scientific community, and the solutions of the engineering problems of mass production, are simple and direct as compared to the processes of changing viewpoints and policy by an electorate.

Have you ever stopped to think how many things taught as facts to us as students are no longer considered correct or of as simple a solution? Then consider that the average adult voter has this same background of misinformation but with no opportunity to update it.

We face water pollution on a scale of which we never dreamed. Fertilizers designed for the production of increased foods to sustain a terrifying growth in world population are polluting our water supplies more insidiously than are organic wastes. Chemists are proposing solutions! But will the citizenry understand these proposals and approve the necessary expenditure of billions of dollars to safeguard our waters for the survival of the human population

and of the biotic community? We no longer talk of pollution as meaning only filthy *water*. We are also greatly concerned that the delicate balance of oxygen production by photosynthesis and oxygen consumption is being seriously threatened by the machines and processes of our modern world. Pollution is taking place not only in water and air but in the soil itself.

The consumption of our resources is accelerating at an alarming rate with crude wide-spectrum harvesting — and, at best, only partial use. Rapidly we are learning that many of the unwanted or wantonly destroyed by-products are more valuable than the prime product itself. Sometimes we are fortunate that the by-products are still available in the piles of waste dumps; but all too often they have been discarded as useless pollutants of our environment.

How often the "practical" manufacturer, engineer, or other consumer has overridden the "conservationist," labeling him as an emotionalist or an impractical dreamer, only to learn in a generation or less that the conservator was more practical than the immediate consumer.

I would not for a moment suggest that we should build no bridges, dams, or roads; plow no fields; cut no trees; spray no insects; devise no new uses of our resources. But I would suggest that the immediate dollar return on a project which consumes or alters our habitat can be, and often is, ultimately more costly to the public than it was profitable to the individual harvester.

In reality, in all this habitat destruction, we tend to forget that it is our own habitat which we are altering at such an alarming rate; and that we are biological creatures living in and depending on biotic factors, no matter how mechanized, commercialized, and synthesized our civilization becomes. Yet, with every struggle to preserve our natural world, the battle lines usually appear to be drawn between the "practical realist" and the "impractical dreamer" in almost direct inversion to the long-term human needs.

As a nation, we need to be conscious of the changing demands of technology on our available resources. Let me illustrate: The American Indian in Illinois made use of very few resources, and he used them very directly. He was dependent on cherts to produce projectile points and sharp-edged cutting and scraping tools, on clay for soft-fired pottery for containers, and on bone and horn for tools. For food he used native game and plants. Only the more recent Indian cultures cultivated corn, amaranth, squash, and beans for food.

Let us follow some of man's changing demands on the geological resources alone. Under French supervision, the Indians who had mined only chert were taught to mine galena. Under French supervision, they smelted the ore in crude earthen smelters, separating the lead which was then transported to Canada for shot to use against the British in the French and Indian War. The British at Jamestown processed wood into charcoal and used it to melt the local sand for producing blown glass. In early times, man harnessed water power to operate lumber and flour mills; later, coal and oil became

sources of energy. The mineral resources harvested in Illinois last year amounted to $500,000,000. In 500 years Illinois has moved from a Stone Age of agriculture through an agricultural economy, an industrial economy, and into a technological economy.

Agriculturally, we are one of the world's leading producers of food. Our land is still a major resource, but we employ less than ten per cent of our manpower to work the land. Five hundred years ago, a single Indian woman planted, cultivated, harvested, stored, and cooked corn, serving her family a simple corn bread as did every other Indian woman of her culture. Two hundred years ago, a few more people were involved in the processing and distribution of the grain, and even in the time of our grandparents most of the bread was still baked at home. By 1935, half of the population had moved from the farms into manufacturing and service tasks. Today, ninety per cent of our people live in urban and suburban America. Economists tell us that we have moved from the Industrial to the Technological Age.

Change in our Technological Age is so rapid that job obsolescence is now the rule. The average worker must retrain for new jobs two, three, and four times during his working career. Even those in specialized scientific and professional fields must keep in constant touch with new findings in their expanding fields to avoid obsolescence. One cannot help but wonder at the rate of depreciation of so many college educations — sometimes expressed as the "half life" of a college degree. Today we face a serious dual responsibility: First, to maintain our respective professional competence through almost constant study and retraining; and, secondly, the constant and appalling task of bringing before the rest of the population a sufficient understanding of this expanding knowledge so that it may be used to insure not only our survival but also the survival of our way of life.

We have heard the alarming reports on the aging of Lake Michigan. In fact, there is no longer a single major stream that is not seriously altered by pollution and silt. Now added to sewage pollution is industrial pollution, fertilizer and insecticide pollution and now thermal pollution by both conventional and atomic plants.

Illinois, though not unique among industrial states, contributes to this pollution not only in the Great Lakes but also through the Mississippi drainage and, thereby, to the Gulf of Mexico. Here, as in other parts of the world, conditions on the continental shelf are apparently becoming less suitable for freeing oxygen by photosynthesis. This, at the very time when our world population and its expanding use of fossil fuels is consuming oxygen at a rate that, according to Dr. Lamont C. Cole, Professor of Zoology at Cornell University, is approaching dangerous levels.

Whether this frightening point of imbalance between CO_2 and O_2 comes in our lifetime or in that of our childrens' children, we nevertheless face a major scientific problem. It will require not only that solutions be found but that the im-

plementation of those solutions be undertaken by governments with the full support and cooperation of their people. Furthermore, these solutions must be undertaken while we still have our wits about us — not in a stupefying low oxygen atmosphere!

I cannot help but remember the strange effect that flying above 12,000 feet had on us in those World War II planes without pressurized cabins. I remember that problems were so simple of solution in the high rarefied atmosphere; but the answers were so often wrong. Yes, we took our ''trips'' before the days of psychodelic drugs.

I recall the story of the veteran Pan American navigator who was lost on a flight from South America to Ascension Island on a cloudy night. He requested his captain to fly above the clouds for a few minutes so that he could get a fix on the stars. As they broke clear above 16,000 feet, the navigator became enthralled by the beautiful sky; but after returning below the 10,000-feet level to adequate oxygen, he suddenly realized that he had completely forgotten to shoot the stars for the much-needed fix. Such was the effect of an inadequate oxygen supply on one of the world's most skillful veteran navigators.

I hope we act before we enter a world of daydreams — act not with scorn of that ''stupid'' public but with the realization that we as members of the scientific community have not completed our task unless we have helped to implement the application indicated by our findings. Even pure science has a dual responsibility: To exemplify the utmost in absolute integrity and to implement the indicated programs, particularly as they apply to our world and its survival. We are no longer allowed the luxury of hiding our findings for posterity behind secret codes and systems—as did Leonardo da Vinci in order to escape burning at the stake. We are fortunate to live in a world that believes in the miracles of science. We must not lose our advantage to the pseudoscientists and the opportunists by default!!

We must exert our every influence in the clarification and support of significant laws and resolutions so that the citizenry will be sufficiently informed to understand and elect the road not only to survival but to a richer, fuller life. This requires, first, interpreting the answers to the problem of world use of resources; and, second, motivating the public to want these solutions. Our role has always been broader than discovery and recording. Like it or not, we also must merchandise our product rather than depend solely on the huckster.

Vocabulary is one of the greatest barriers between the specialist and the public. Every field of science, trade or profession has its own vernacular, a combination of technology and verbal shorthand. When we seek to explain an idea to others, we must in the main use their vocabulary in speaking or writing. In the museum field, this is a most difficult challenge facing museum curators: expressing a complex idea in simple understandable language and in a straightforward, noncondescending manner when preparing a label, writing a popular article, or appearing on television. I suggest that communication with the non-

scientists is a major problem and a responsibility of the entire scientific community.

Recently I received a most appropriate communication from John H. Melvin, President of the Academy Conference of the American Association for the Advancement of Science, in which he reported on a request for a National Science Foundation grant to set up a workshop conference for editors of the forty-four state and local academies. The major problem being considered is the analysis of current publications of the various academies. In most cases these publications account for over one-half of each academy's budget. The majority of the members prefer to publish their most significant reports in the journals of their respective disciplines, for comparatively few of the academy journals are abstracted in the principal abstract sources. We are fortunate that the *Transactions of the Illinois State Academy of Science* is so widely abstracted. For this we owe a debt to both our authors and our editors. Yet, lest we get too smug, our journal really reflects the publication policies of the 40's and 50's.

As Dr. Robert E. Gordon, Secretary of the Council of Biology Editors, expressed it before the Academy Conference in 1966: "While the general role of the Academy has moved from its pre-1940 monotypic function as a major forum for the discussion of original research in all fields to a mid-1960 polytypic function — perhaps best summed up in the words 'educational catalyst' — I strongly suspect that the publication practices of the majority, through neglect, still attempt to reflect the pre-1940 picture. A reconciliation of these aspects, in those organizations where it is needed, would not only serve to enhance the publication activities and image of the Academy, but also would contribute in a very substantial way to the resolution of major problems facing the whole scientific community."

Perhaps we should devote at least one session of each section at our Annual Meeting to a panel type discussion which would bring some major facet of discovery or thought from the national picture as an updating forum for those members who could not attend their respective national forums. This would help to better establish our local lines of communication, and it would, I feel, improve the drawing power of our Academy.

The next logical step would seem to be to break the tradition of publishing only new research in our *Transactions* and, perhaps, to expand the "News and Notes" to include important world-wide developments in the various scientific fields.

As we improve the flow and modernize the functions of our communications, we will not only benefit but we will also become more articulate locally. I firmly believe that the single most important key to public understanding is better, broader, more general improvement of our internal communication. Let us, among ourselves, consciously reverse the trend of never finding that elusive "something" we so often feel is lacking at these important gatherings. Let us establish that necessary

communication with the fascinating frontiers of our work — in order to rekindle the *spark,* the *challenge,* and the *enthusiasm* that drew us originally to this intriguing world of science. Let us then use our enthusiasm to stimulate a renewed interest and understanding of this important realm of public understanding of science! Then and *only* then will *we,* as portions of the scientific community, be making *our* contribution to the necessary understanding of the world of science in a democracy!

DEVONIAN STRATIGRAPHY AND BIOSTRATIGRAPHY AT DUTCH JOHN MOUNTAIN, LINCOLN COUNTY, NEVADA

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ABSTRACT. — The Devonian sequence at Dutch John Mountain includes the Silverhorn Dolomite (Lower Dolomite and Limestone Member—1217 feet, Middle Limestone and Dolomite Member—470 feet, and Upper Dolomite Member—778 feet), West Range Limestone—235 feet, and the Lower Member of the Pilot Shale — 122 feet. Conodonts and other invertebrate fossils indicate that the Silverhorn Dolomite ranges from the latest Givetian through the earliest Fammenian, and that the West Range Limestone is entirely Fammenian as well as the Lower Member of the Pilot Shale. The base of the sequence is covered and the Lower Member of the Pilot Shale is disconformably succeeded by the Joana Limestone of Mississippian age.

The Devonian rocks at Dutch John Mountain were designated a reference section for the Silverhorn Dolomite and West Range more than thirty years ago but have yet to be described in detail adequate for stratigraphic comparison. This study is intended to provide such a description as well as a more precise biostratigraphic correlation.

Dutch John Mountain is in northern Lincoln County, Nevada (T27N, R65E), about 70 miles south of Ely and about 40 miles north of Pioche (Fig. 1). The mountain is an isolated north-south trending mass of Devonian and Mississippian rocks about 3x5 miles and rising about 2500 feet above surrounding Lake and Dry Lake Valleys. These valley floors are formed of Quaternary debris at an approximate average elevation above sea level of 6500 feet.

Strata in the mountain strike with an average value approximately N 65° E and dip generally southeast about 12°. Thus, the oldest Devonian rocks crop out at the north end of the mountain and successively younger rocks are exposed southward. Devonian Silverhorn Dolomite comprises the bulk of the mountain and is succeeded by relatively thin layers of Devonian West Range Limestone and the Lower Member of the Pilot Shale. At the top of the mountain Mississippian Joana Limestone crops out in prominent cliffs and on a south-sloping dip surface. Scattered patches of Peers Spring Formation occur also on the mountain top, and this formation along with Chainman Shale and Hamilton Canyon Formation form a strike valley separating Dutch John Mountain from Grassy Mountain to the south. The Dutch John Mountain block is a relatively simple homoclinal mass broken by minor faults belonging to two sets trending roughly WNW and NE. In addition a prominent NNE trending fault bounds the west side of the mountain at the south end whereas the northern point of the range is complexly faulted, brecciated and, in part, bleached.

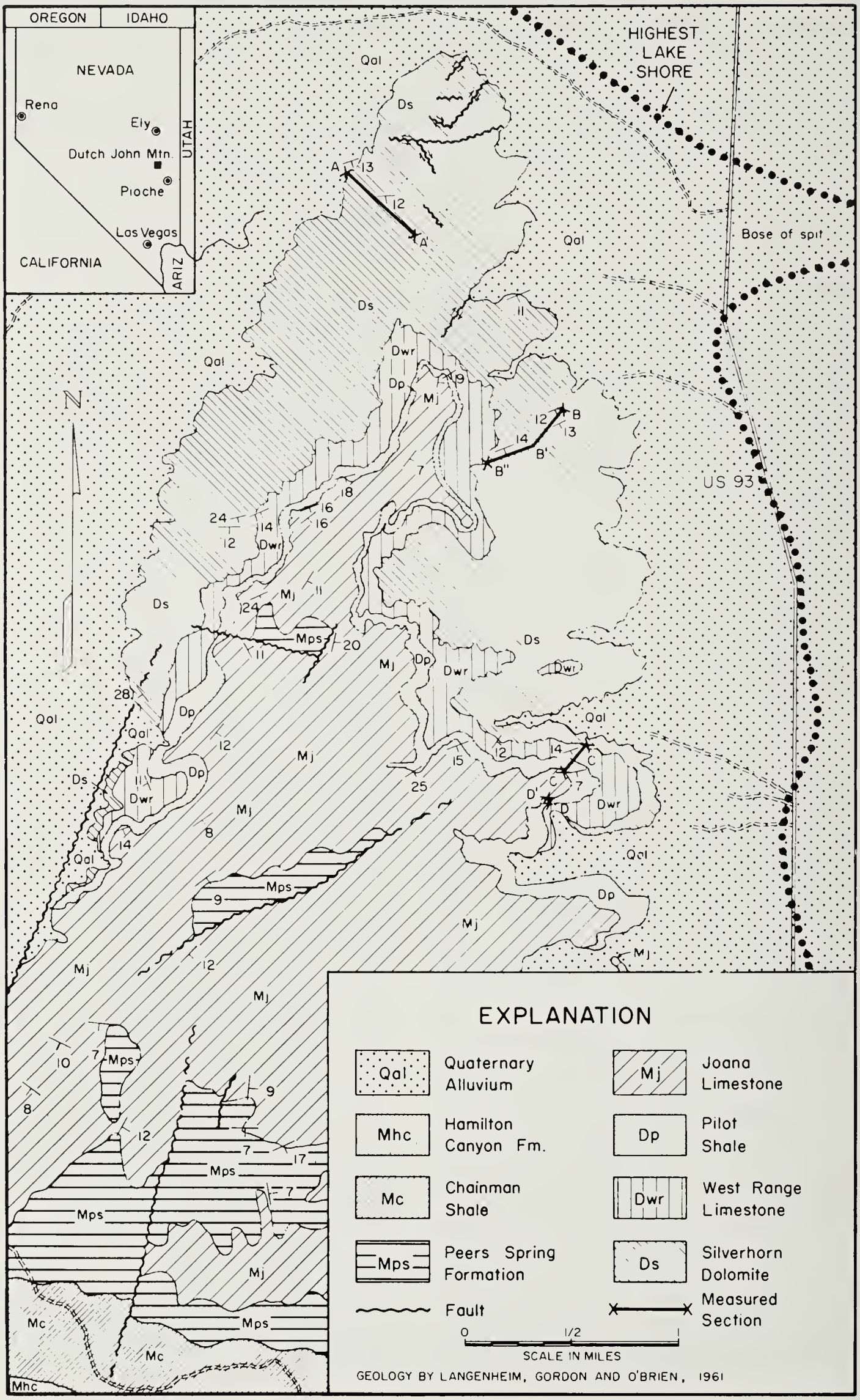

FIGURE 1. — Geologic map of Dutch John Mountain.

Field work for the project was done during the summer of 1961. O'Brien and Gordon located and measured stratigraphic sections (Fig. 1 AA', BB', CC', and DD'). During the last month of field work Langenheim examined the measured section traverses and verified and extended geologic mapping. Mapping was done in the field on aerial photographs with compilation of the published map using an uncontrolled mosaic as a base. Sections were measured by the Jacob's staff method.

Physical Stratigraphy

PREVIOUS WORK: Westgate and Knopf (1932) first assigned the Devonian rocks of Dutch John Mountain to the Silverhorn Limestone and West Range Limestone following their original description of these units in 1927 (Westgate and Knopf, 1927, p. 822). The type section for the Silverhorn Dolomite, however, is at the old Silverhorn mining camp 4 miles northwest of Bristol Pass and that of the West Range Limestone is in the nearby West Range. Westgate and Knopf (1927, 1932) assign between 500 and 600 feet of thin- to medium-bedded, partly nodular limestone weathering a characteristic yellow to the West Range Limestone at Silverhorn and in the West Range. Langenheim (1960), however, has redescribed the type section and reports it approximately 300 feet thick. Westgate and Knopf (1927, 1932) describe a thin quartzite unit, capping the Silverhorn Dolomite in the West Range and at Silverhorn, which they consider a division between Middle and Upper Devonian rocks. The bulk of the Silverhorn Dolomite, however, is described as "3000 feet of gray and brown thick and thin-bedded limestones and dolomites. . ." A characteristic brown dolomite, filled with small rod-shaped corals, is termed "Spaghetti limestone."

In addition to the units named by Westgate and Knopf at Pioche other formations described in the Eureka and White Pine Mining Districts, the Robinson Mining District near Ely, and, in the Gold Hill District are of nomenclatorial significance in connection with the Dutch John Mountain sequence. The Nevada Limestone, originally described in the Eureka and White Pine Mining Districts by King (1878) and Hague (1892), has since been revised by Merrim (1940) and by Nolan, Merriam and Williams (1956). These rocks are now assigned, in ascending order, to the Beacon Peak Dolomite, Oxyoke Canyon Sandstone, Sentinel Mountain Dolomite, Woodpecker Limestone and Bay State Dolomite Members of the Nevada Formation; and, the Meister Limestone and Hayes Canyon Limestone Members of the Devils Gate Formation. Limestone dominates the sequence west of Eureka with increasing proportions of dolomite and decreasing amounts of quartzite toward the east. The Nevada Formation and Devils Gate Formation are approximate lithologic and time-rock equivalents of the Devonian carbonates at Dutch John Mountain. The Pilot Shale, originally described by Lawson (1906), was named by Spencer in 1917 with a type section in the Robinson (Ely) Mining District. This widespread unit succeeds and is apparently wholly younger than the Devonian carbonate sequence of the eastern rGeat Basin. Nolan (1935) described the Sevy Dolomite, Simonson Dolomite and Guilmette Formation in the Gold Hill District, assigning all units to the Middle Devonian.

At Dutch John Mountain, Westgate and Knopf (1932, p. 19) describe 2800 feet of Silverhorn Dolomite, of which more than 2000 feet are referred to as "limestone". In addition, 330 feet of West Range Limestone are recognized. Kirk's (1927) report of a single *Stringocephalus* sp from a loose block of limestone at the northern foot of the mountain, along with occurrences of *Stromatopora, Cladopora, Favosites*, and *Atrypa reticularis* led Westgate (Westgate and Knopf, 1932) to assign the Silverhorn Dolomite to the Middle Devonian. Late Devonian fossils, including *Athyris angelica, Pugnax pugnus, Camarotoechia contracta* and *Spirifer whitneyi*, were reported from the West Range Limestone (Westgate and Knopf, 1932). Westgate and Knopf's (1932) reconnaissance study does not mention faulting and thus the 2800 feet of Devonian rocks reported at Dutch John Mountain probably includes the faulted and brecciated material at the north end of the mountain.

Merriam (1940, p. 39), in a brief dis-

cussion using both lithologic and faunal criteria, defines zones "A" through "E" within the uppermost 1935 feet below the Joana Limestone at Dutch John Mountain. Below these rocks an additional 1200 feet of rock are correlated with the "Silverhorn Dolomite" of Westgate and Knopf. Zones A and B reportedly contain *Cyrtospirifer* sp., and Merriam (1940, p. 39) believed them at least partially correlatable with the West Range Limestone of Westgate and Knopf. Zone C, 750 feet of "light and dark-colored limestone which is probably dolomitic, and which weathers blocky and cliffy.", is questionably assigned (Merriam, 1940) to the Late Devonian because it contains *Cladopora* sp. and stromatoporoids. Zone D, 300 feet of "dark, mottled, argillaceous to shaly limestone" (Merriam, 1940, p. 40) is stated to contain a well-preserved late Devonian or early Carboniferous brachiopod fauna. Zone E, 85 feet of "thin-bedded, platy, gray shales" is reported conformably succeeded by the "Carboniferous limestone." Merriam (1940) places the Devonian-Mississippian contact somewhere with zones C and D and considers zones C, D, and E to be younger than the West Range Limestone and older than the Bristol Pass (Joana) Limestone of Westgate and Knopf. According to Merriam, the greatest amount of the lowest 2000 feet of Devonian rock at Dutch John Mountain is to be correlated with the Devils Gate Formation of the Roberts Mountain Quadrangle.

Tschanz (1960, p. 202) assigns the Devonian rocks at Dutch John Mountain to Nolan's (1935) Middle Devonian Guilmette Formation and Spencer's (1917) Pilot Shale. The Guilmette Formation of Tschanz (1960), therefore, includes the Silverhorn Dolomite and part of the West Range Limestone of Westgate and Knopf (1932). It includes also all Devonian rocks assigned to Merriam's zone C and the units below. Tschanz, however, assigns Merriam's zones D and E, or the upper 380 feet of Devonian at Dutch John Mountain, to the Pilot Shale. Tschanz's Pilot Shale at Dutch John Mountain is equivalent in part to the West Range Limestone and reportedly rests on, or includes, an unconformity. Referring to Merriam's (1940, p. 39-40) description, Tschanz assigns the Guilmette Formation at Dutch John Mountain to a limestone facies, one of three facies employed by him in discussing the Guilmette Formation of Lincoln County.

Stratigraphic Terminology: The Silverhorn Dolomite concept is herein employed in preference to the Guilmette or Devils Gate Formation, because the rocks at Dutch John Mountain, though biostratigraphically and temporally equivalent to these units, appear lithologically distinct. The uppermost 235 feet of Devonian Limestone at Dutch John Mountain is referred to the West Range Limestone as described in the type area (Westgate and Knopf, 1932; Langenheim, 1960). At both localities this limestone is a well-marked lithologic unit and is readily distinguished from the underlying Silverhorn Dolomite and succeeding Pilot Shale or Joana Limestone. The Lower Member of the Pilot Shale is recognized at Dutch John Mountain in the sense of Langenheim (1960).

Three lithologically defined members within the Silverhorn Dolomite are not given formal status because their regional significance is doubtful. They constitute a lower member of dolomite with interbedded limestone, a middle limestone member with interbedded dolomite, and an upper dolomite member.

Basal Contact: On Dutch John Mountain the base of the Devonian sequence is unexposed and the thickness of hidden Devonian rock is unknown. Kellogg (1960)reports about 4860 feet of pre-West Range Limestone Devonian rock at Sunnyside about 20 miles west-south west and, from this, it may be estimated that perhaps as much as 2400 feet of Devonian rock are covered at the north end of Dutch John Mountain. Another estimate may be based on Kirk's report of a single specimen of *Stringocephalus* sp. in float at the north end of the mountain. In this region, *Stringocephalus* characteristically forms a single biostrome in the Simonson Dolomite. About 27 miles northwest, Kellogg (1960, 1963) notes this biostrome about 2100 feet above the base of the Devonian System at Sixmile Ranch, Sec. 10, T. 10 N., R. 62 E. He (Kellogg, 1963, p. 698) correlates the Sixmile Ranch occurrence and the lowest exposed Devonian rocks at Dutch John Mountain in his "upper alternating member of the Simonson Dolomite." Thus the hidden section at Dutch John Mountain reasonably appears to be somewhat more than 2,000 feet thick. Kirk's observation, however, has never been duplicated and the present authors found no rock on Dutch John Mountain uniquely charac-

teristic of the Simonson Dolomite of eastern Nevada. We have collected conodonts, however, from the lowest Devonian exposed on the mountain which appear to be Givetian; the approximate age of the eastern Nevada *Stringocephalus* occurrences.

Lower Dolomite and Limestone Member, Silverhorn Dolomite: Units 1 to 59 (Figs. 2-4) consisting chiefly of thin-bedded to massive dolomite comprise the Lower Dolomite and Limestone Member of the Silverhorn Dolomite. This member is 1217 feet thick. The dolomite weathers medium brown-grey or light brown-grey to light grey in alternating layers. Fresh rock is somewhat darker. Among the many thin limestone interbeds unit 14 and units 26 to 29 comprise thicker limestone sequences. In addition are to be noted two layers of dolomite breccia, units 32 and 42, and one of limestone, unit 20.

Units 14 and 16, both limestone, contain relatively abundant invertebrate fossils, including *Atrypa* sp., *Cyrtospirifer* sp., *Productella* sp. and, in unit 16, *Pachyphyllum* sp. Conodonts were recovered from units 9, 14 and 29.

Insoluble residues range from 0.1% to 12.0% in the dolomite units and from 0.2% to 9.5% in the limestone samples. In general, however, the dolomite appears to have more insoluble content. The dolomite breccia reaches a high of 16.5% insoluble residue.

Middle Limestone and Dolomite Member: Units 60 to 40 (Fig. 4) consist chiefly of thin-bedded to massive, well laminated limestone and comprise the 470 feet of rock assigned to the Middle Limestone and Dolomite Member. The limestone is almost black on fresh surfaces but weathers medium-gray to dark brown-gray. It is microcrystalline and much of it is pelletoidal or particulate. Most dolomite layers are medium to dark brown-gray and weather light gray to medium, brown-gray.

Limestone insoluble residues range from 0.3% to 10.9%, those of the dolomite range from 0.8% to 39.4%, and those of dolomitic limestone from 0.7% to 14.0%.

Units 63 and 64, both limestone, contain relatively abundant and well-preserved invertebrate fossils, including *Atrypa* sp. *Douvillina maxima*, "*Cyrtina*" sp., "*Cyrtospirifer*" sp., *Leptostrophia fragilis*, "*Productella*" sp. and, in unit 64, an arthrodire plate. In addition, a well-preserved conodont, *Palmatolepis glabra pectinata* (Ziegler) was recovered from unit 79.

Upper Dolomite Member, Silverhorn Dolomite: Units 91 through 106 (Figs. 4-5) consist chiefly of massive to thin-bedded dolomite with minor sandstone and comprise the 778 feet of rock assigned to the Upper Dolomite Member. The dolomite is mostly dark to light brown and weathers light brown to gray brown. In the upper portion layering and lamination are prominent. Unit 106 and portions of units 104, 102 and 99 are comprised of alternating light and dark colored layers as much as one foot thick and units 96, 98 and 99 display light and dark colored laminae about 1/10 inch thick. Units 100 and 103 are 6 inch layers of quartzite and sandstone respectively. Adjacent portions of units 99 and 103 and all of unit 101 also are notably sandy.

Insoluble residues, excepting those of units 99, 101, and 103 which are sandy, and of units 92 and 94, which contain silicified fossils, are generally small and comprised of quartz silt, clay, and iron oxides. An organic scum was produced during etching of samples from units 92 and 95.

Silicified brachiopods from unit 92 include *Atrypa reticularis, Tenticospirifer* cf. *T. keleticus, T. utahensis, Cyrtospirifer* cf. *C. whitneyi* and *Pugnax* (?) sp. Poorly preserved brachiopods, gastropods, rugose corals and *Cladopora* sp. occur widely in the member and conodonts recovered from units 92, 93, 94, 95, 96, 97, 101, 102, 105, and 106 are listed in figure 7.

West Range Limestone: Units 107, 108 and 109 (Fig. 6) consist of limestone and argillaceous limestone and comprise the 235 feet of rock assigned to the West Range Limestone. Units 107 and 108 are of cliff-forming fine to medium-grained, brown limestone which weathers gray to buff. Unit 109 consists of fine-grained brown to black limestone forming a series of benches and ledges of more thinly bedded and locally nodular argillaceous rock and more pure limestone. All weather gray to yellow-buff color.

Insoluble residues from the cliff-forming limestone consist of silicified tubules, clusters of quartz anhedra and minor hematite. Residues from unit 109 also contain clay and, locally, silt.

Scattered solitary corals occur at the base of unit 107 and the basal 8-inches of unit 108 is an ostracode coquina containing abundant *Cyrtospirifer* cf. *S.*

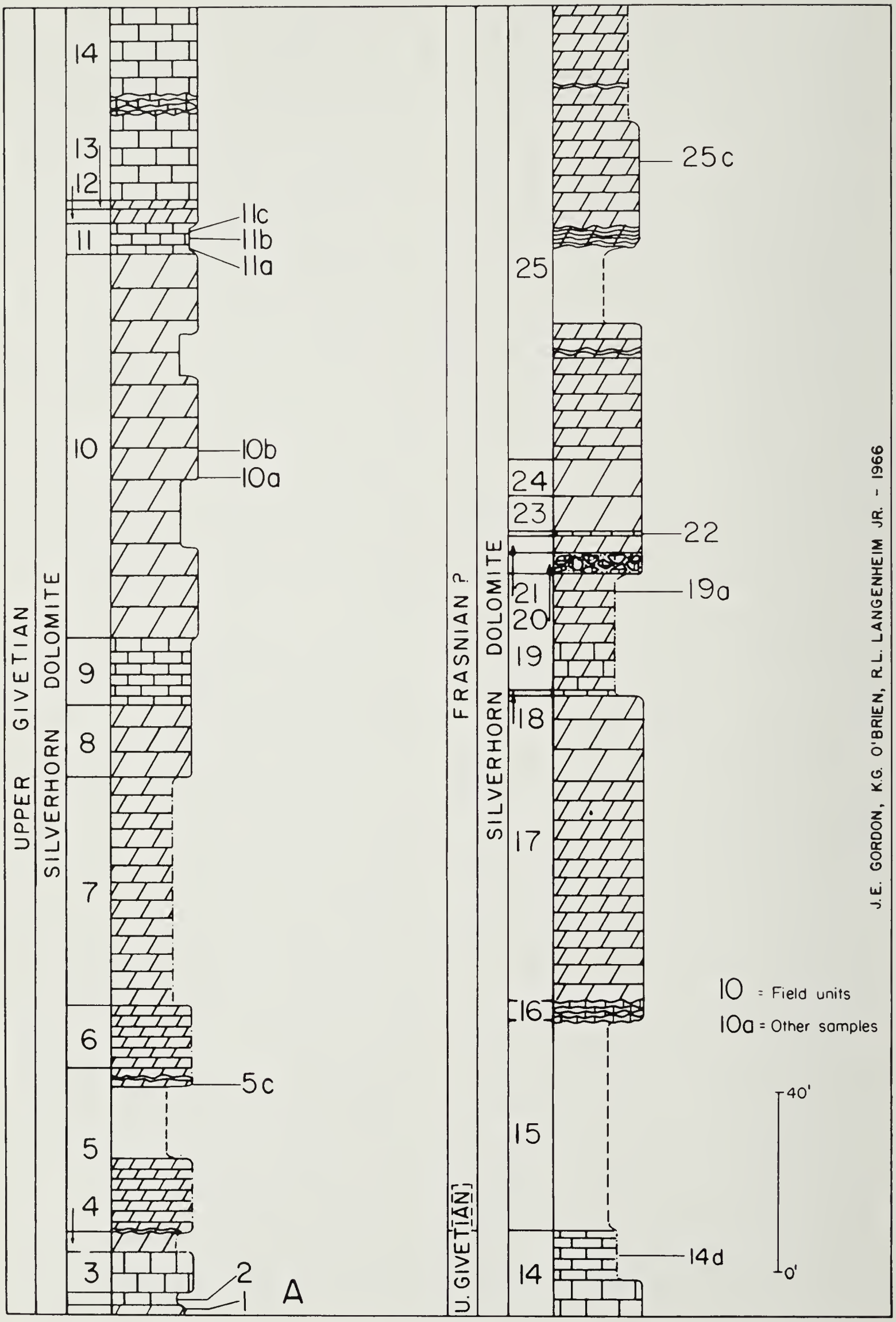

FIGURE 2. — Columnar section, units 1-25, Dutch John Mountain.

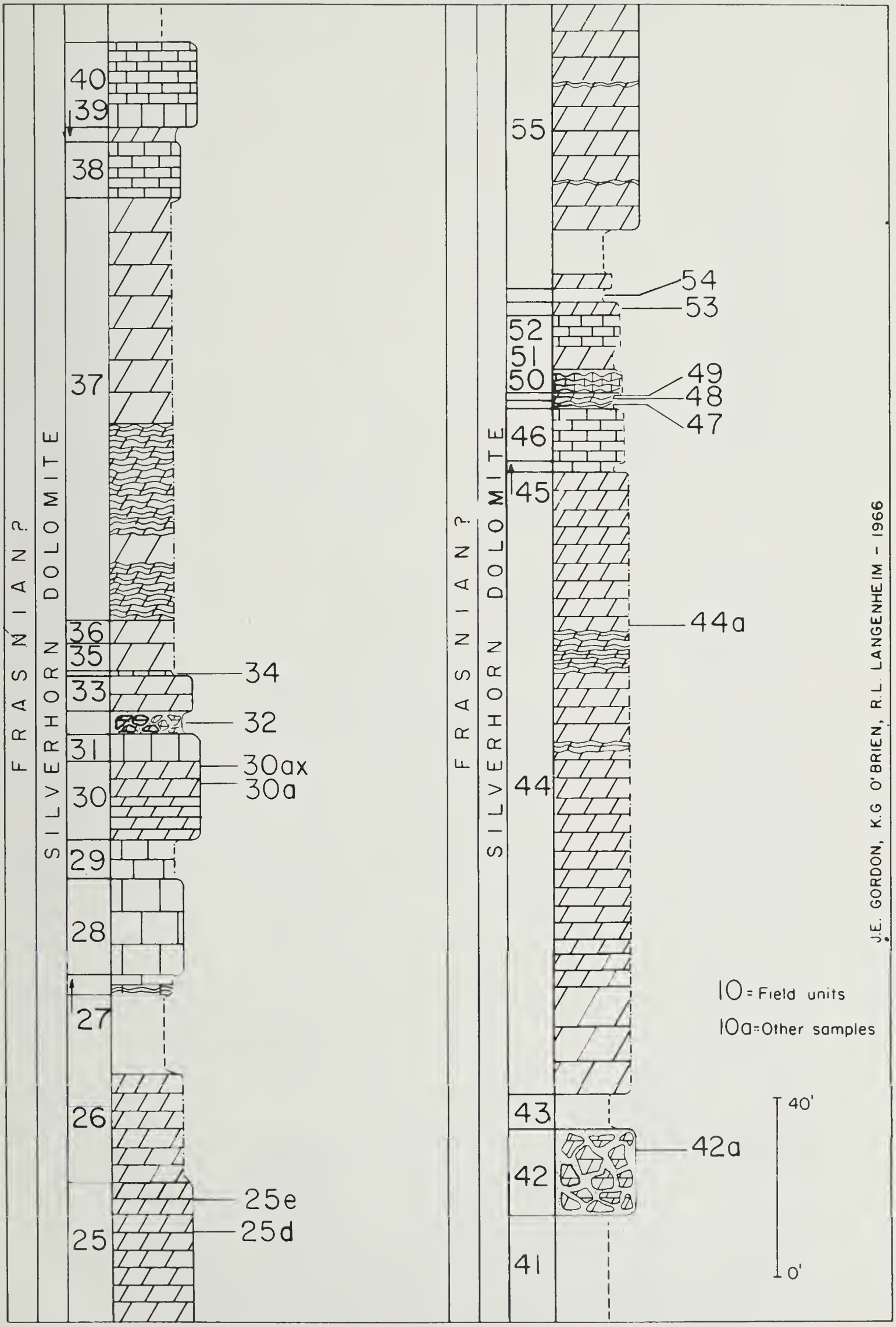

FIGURE 3. — Columnar section, units 25-55, Dutch John Mountain.

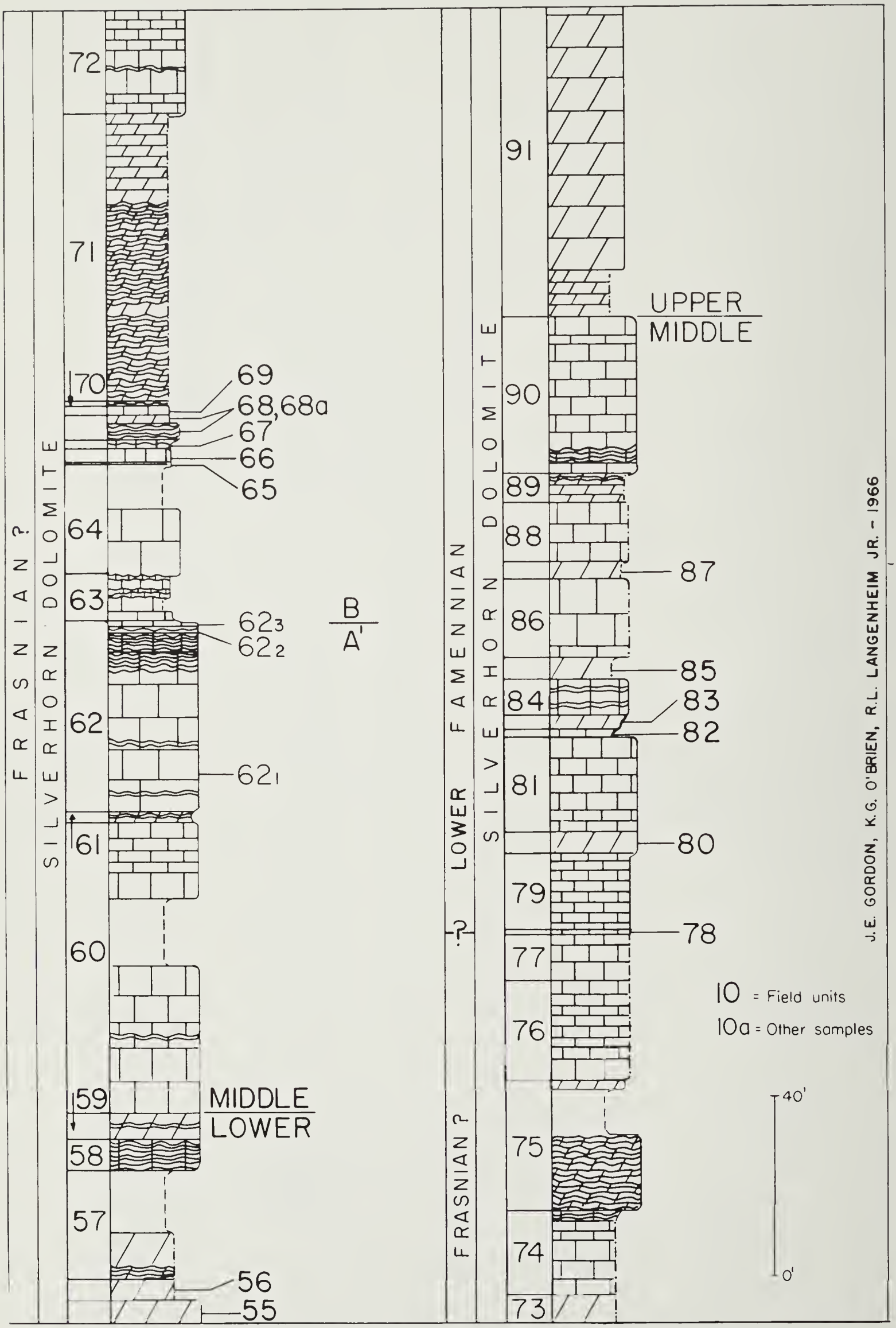

FIGURE 4. — Columnar section, units 55-91, Dutch John Mountain.

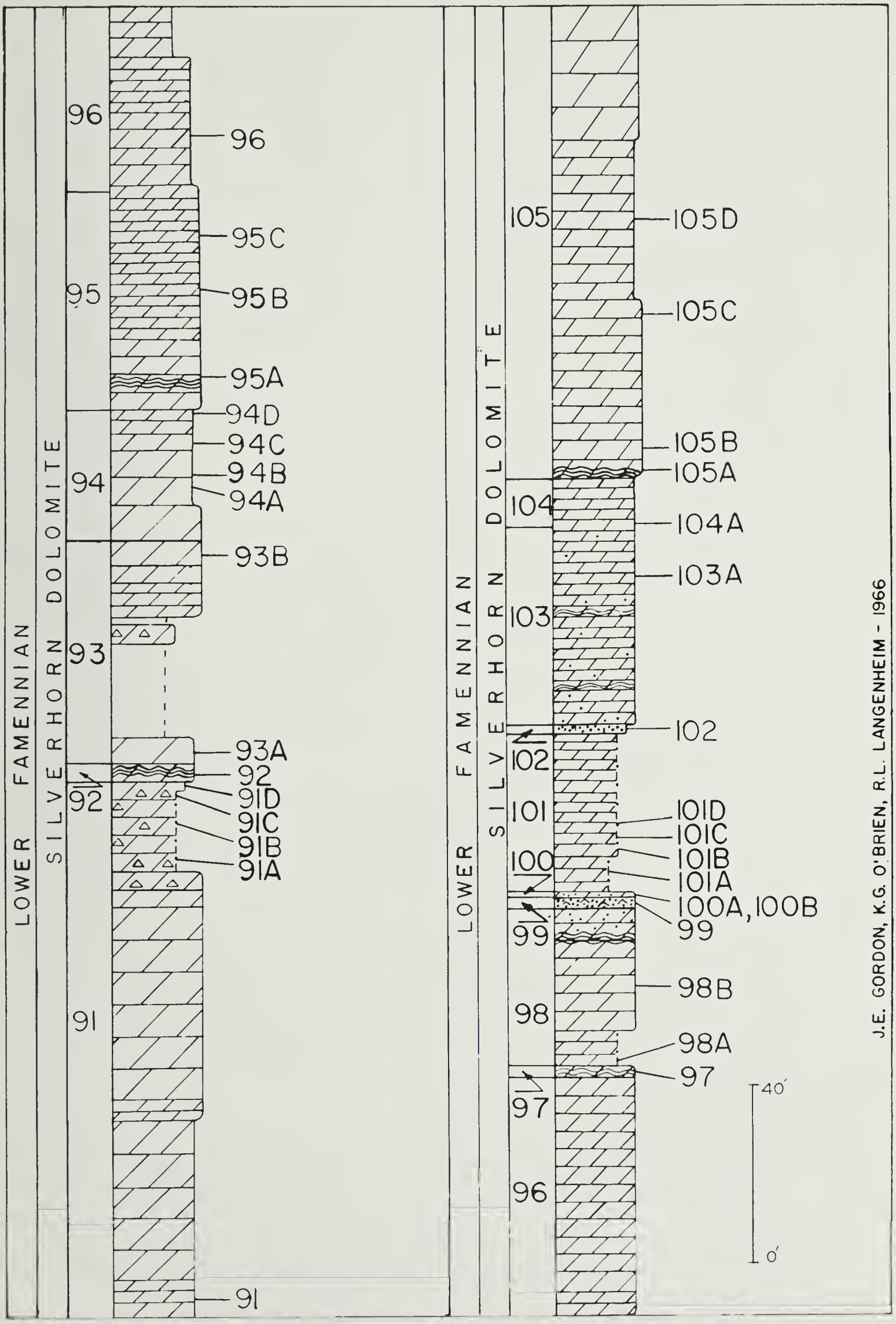

FIGURE 5. — Columnar section, units 91-105, Dutch John Mountain.

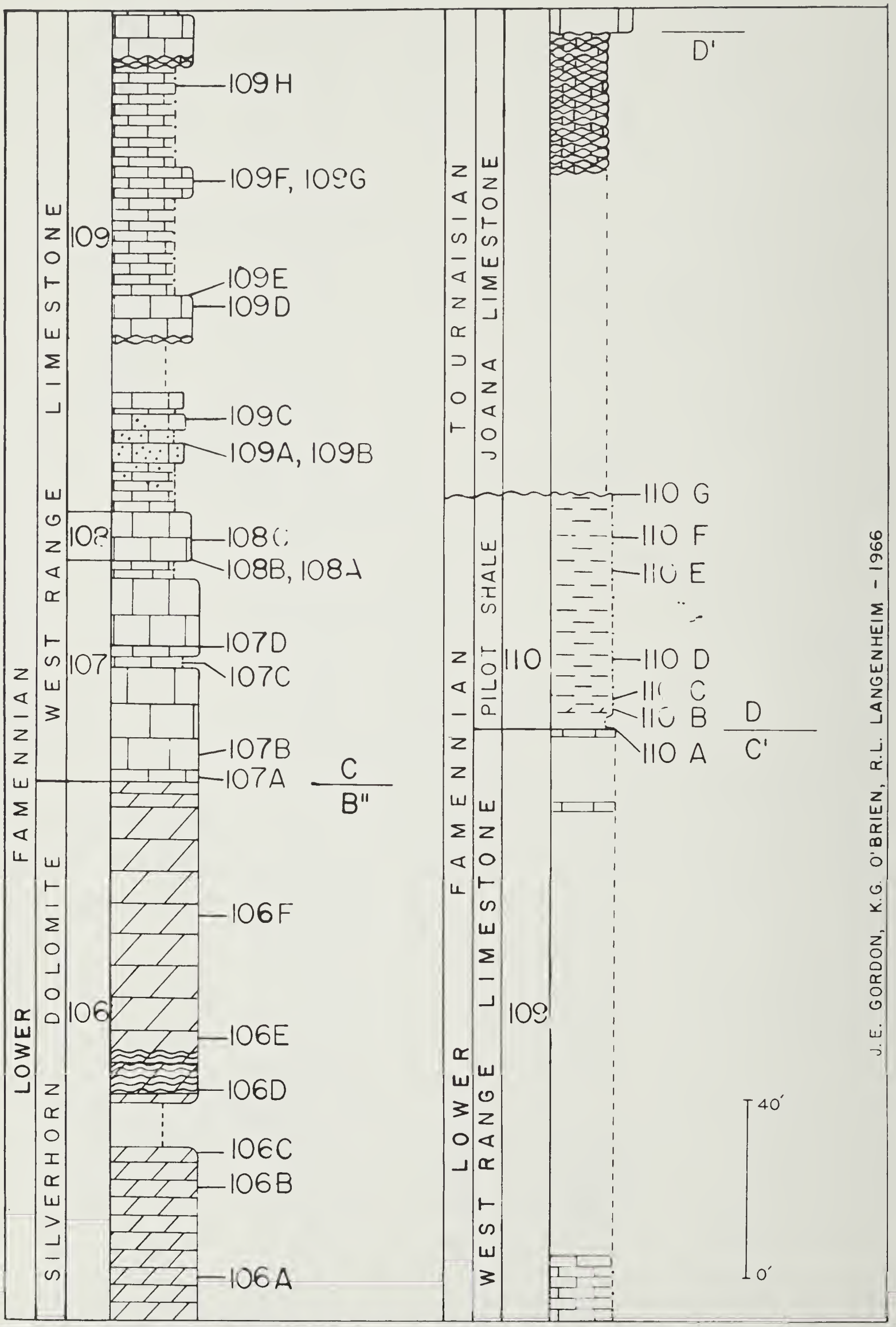

FIGURE 6. — Columnar section, units 106-110, Dutch John Mountain.

FORMATION	SILVERHORN DOLOMITE												WEST RANGE LIMESTONE			PILOT SHALE			
MEMBERS																			
COLLECTIONS / FOSSILS	9	14	29	60	79	92	93	94	95	96	97	101	102	105	106	108	109	110	111
Hindeodella sp.	X	X		X		X											X		
Lonchodina ? sp.			X																
Ozarkodina elegans (Stauffer)		X																	
Palmatolepis glabra pectinata Ziegler					X														
Polygnathus sp.		X																	
Polygnathus varca Stauffer	X	X																	
Falcodus sp.						X											X		
Neoprioniadus sp.						X													X
Centragnathodus delicata (Branson and Mehl)						X													
Icriodus alternatus Branson and Mehl						X													
Palmatolepis triangularis Sanneman						X													
Polygnathus webbi Stauffer						X													
Polygnathus triangularis Branson and Mehl						X											X		
Palmatolepis glabra Ulrich and Bassler																		X	
Palmatolepis cf. P. rhomboidea Sanneman										X									
Polylaphodonta sp.															X				
Palmatolepis superlobata Branson and Mehl																	X		
Scutulo ? sp.																	X		
Palmatolepis delicatula Branson and Mehl																	X		
Lonchodino? sp.																	X		
Palmatolepis rhomboidea Sanneman																	X		
Palmatolepis quadrantinodosa marginifera Ziegler																	X		
Hibbardella sp.																	X		
Apotognothus sp.																	X		
Palmatolepis quadrantinodosalobata Sanneman																	X		
Palmatolepis inflexa Müller																	X		
Polygnathus semicostata Branson and Mehl																	X	X	
Palmatolepis sp.														X					X
Palmatolepis tenuipunctata Sanneman																	X		
Apatognatus varians Branson and Mehl																	X		
Palmatolepis bet. *P. rhomboidea* and *P. quadrantinadosa*							X												
AGE	GIVETIAN ?					FRASNIAN ?				LOWER FAMENNIAN									

FIGURE 7. — Checklist of Devonian conodonts from Dutch John Mountain.

whitneyi. Unit 109 contains abundant brachiopods, among which are *Camarotoechia contracta*, *C. congregata* (?), *Athyris angelicoides*, *Tenticospirifer* cf. *T. keleticus* and *Cyrtiopsis* (?) sp. Conodonts, listed in figure 7, are also abundant in unit 109.

Pilot Shale: Units 110 and 111 (Fig. 6) consist of dolomitic mudstone and platy, silty, calcareous shale and comprise the 122 feet of rock assigned to the Pilot Shale. Unit 110 consists of gray silty dolomitic mudstone and unit 111 of platy, gray brown calcareous siltstone in layers about 1/16 inch thick. Insoluble residues of the mudstone are dominated by porous masses of quartz subhedra. In contrast those of the siltstone include significant quantities of broken hornblende prisms in quartz silt.

Polygnathus semicostata and *Palmatolepis glabra* occur in unit 110 and *Neoprioniodus* sp. and *Palmatolepis* sp. occur in unit 111.

Biostratigraphy

Figure 7 summarized the occurrence of conodonts collected from the Devonian sequence at Dutch John Mountain in the course of this study and identified by C. W. Collinson.

Conodonts occurring in units 9 and 14 include *Polygnathus varca* Stauffer but not specimens of *Palmatolepis* or *Ancyrodella*. Consequently these rocks tentatively have been assigned to the latest Givetian.

Unit 15 is covered, but unit 16 contains *Pachyphyllum* sp., a coral known only from Late Devonian rocks in North America. Thus it is concluded that the Givetian-Frasnian boundary probably is within unit 15.

Unit 79 has yielded a single, well-preserved specimen of *Palmatolepis glabra pectinata* Ziegler. This species, according to Ziegler (1962, p. 28-30, 59), ranges through the *rhomboidea* zone and into the *quadrantinodosa* zone or to $II\alpha$ - to $II\beta$, which is the lower Fammenian. This occurrence is not compatible with occurrences of Frasnian fossils in unit 92 and of fossils of comparable Fammenian range in units 93, 109, and 110.

Unit 92 contains *Palmatolepis triangularis* Sanneman, *Polygnathus triangularis* Branson and Mehl, and *Polygnathus webbi* Stauffer. Ziegler (1962, p. 85) reports *P. triangularis* ranging from the Lower *triangularis* zone through the Lower *crepida* zone and possibly into the Middle *crepida* zone or from toI to the lowest toII which is late Frasnian to earliest Fammenian. Collinson (1962, p. 16) reports *P. triangularis* as characteristic of his *Palmatolepis proversa-Palmatolepis triangularis* assemblage zone occurring in the Sylamore Sandstone of the Mississippi Valley. He correlates this assemblage with the $toI\beta$ - toI_τ subzones of the Frasnian. *Polygnathus webbi* Stauffer is probably similar to *P.* sp. aff. *P. webbi* Stauffer as reported by Clark and Ethington (1966, p. 673, tab. 6; p. 684) from the Great Basin Middle and lower Upper Devonian. *Polygnathus triangularis* Branson and Mehl, however, is characteristic of Collinson's (1962, p. 18) *Spathognathus aculeatus* assemblage zone. This zone, widespread in the middle part of the Saverton Formation of the Mississippi Valley, is considered to correlate approximately with the uppermost toV and lowermost toVI zones of the early Strunian of western Europe. Thus, this fossil is not compatible with its apparent associates of Frasnian aspect at Dutch John Mountain.

Unit 93 contains a single undescribed species of *Palmatolepis*, intermediate in character between *P. rhomboidea* Sanneman and *P. quadrantinodosa* Branson and Mehl. Thus, this unit must be as old as the toII subzone of the *Cheiloceras* Stufe as recognized by Ziegler (1962), may be as old as the $toII\alpha$ subzone, and is Fammenian. The Fammenian - Frasnian boundary, therefore, is placed at the contact between units 92 and 93.

Units 94 through 108 lack significant conodonts, but unit 109 contains a large and varied assemblage of Fammenian aspect. Of these; *Palmatoplepis rhomboidea* Sanneman, *P. quadrantinodosa marginifera* Ziegler, *P. quadrantinodosa inflexa* (Muller), *P. quadrantinodosa-lobata* Sanneman and *P. superlobata* Branson and Mehl have a combined range from the base of Ziegler's (1962) *triangularis* zone through the top of the upper *quadrantinodosa* zone which is latest Frasnian ($toI\delta$) to Middle Fammenian or lower *Platyclymenia* Stufe ($toIII\alpha$). *Apatognathus varians* Branson and Mehl also occurs in unit 109, but appears out of place. Collinson (1962) reports this species as characteristic of his *Gnathodus* n. sp. A. assemblage zone in the uppermost Saverton Formation and equivalent parts of the lowermost Louisiana Limestone of the Mississippi

Valley. Collinson considers this zone equivalent to the upper part of the toVI zone of the Strunian.

Palmatolepis glabra Ulrich and Bassler, occurring in unit 110, is reported from Collinson's (1962, p. 16) *Palmatolepis quadrantinodosa-Palmatolepis glabra* assemblage zone in company with *P. rhomboidea* Sanneman and *P. quadrantinodosa* Branson and Mehl. This assemblage zone occurs in the Grassy Creek Formation of the Mississippi Valley and is considered equivalent to the toI$_\delta$ through toIII$_\alpha$ zones, or latest *Manticoceras* Stufe through earliest *Platyclymenia* Stufe in the latest Frasnian to early late Fammenian of Europe. Ziegler (1962, p. 58-59) reports the combined ranges of several subspecies of *P. glabra* as including the upper *crepida crepida* zone through, questionably, the upper *velifera* zone. Thus, unit 110 appears equivalent to the early portion of the *Platycymenia* Stufe or to the early late Fammenian.

Clark and his associates (Clark and Becker, 1960; Clark and Ethington, 1966; Beach, 1961) present a reconnaissance conodont zonation for the Great Basin Devonian. Unfortunately, however, there is little in common between the faunas reported by them and those obtained by us at Dutch John Mountain. *Polygnathus webbi* Stauffer is the only species we have that is comparable to any reported by them from the Middle Devonian, but our examples of this taxon are from the early Frasnian.

Clark and Becker's (1960) zone A is correlated by them with the upper part of the *Manticoceras* Stufe and would thus be approximately equivalent to our fauna from unit 92. Our collections, however, do not include any taxa reported by Clark and Becker.

Clark and Becker assign their zone B to the lowest *Cheiloceras* Stufe or uppermost *Manticoceras* Stufe. Their list of characteristic species includes *Palmatolepis quadrantinodosalobata* Sanneman and *P. superlobata* Branson and Mehl which also occur in our unit 109.

Zone C is considered of late *Cheiloceras* Stufe to probable early *Platyclymenia* Stufe age by Clark and Becker (1960, p. 1666 *not* p. 1665, fig. 2 or p. 1668, fig. 3) and is assigned a range of late *Cheiloceras* Stufe or early *Platyclymenia* Stufe by Beach (1961, p. 44; *not* p. 42, fig. 2). Neither of these authors cite any species from zone C which are also present in our collections, although Zone C is assigned an age approximately equivalent to our units 109 and 110.

Zone D tentatively is assigned to toV or toVI by Beach (1961, p. 44) and contains no species occurring in our collections. Zone E (Beach, 1961) is Early Carboniferous and definitely younger than any materials described in this study.

The above analysis indicates that the Silverhorn Dolomite at Dutch John Mountain ranges in age from probable latest Givetian to earliest Fammenian. Moreover, the rich conodont fauna of the West Range Limestone, as well as the abundance of *Tenticospirifer* in the absence of *Cyrtiopsis*, indicates that this formation is entirely early Fammenian. Also, the conodonts of the Lower Member of the Pilot Shale are of early Fammenian age. Thus, Middle and Late Fammenian rocks apparently do not occur at Dutch John Mountain, because the overlying Joana Limestone is generally considered entirely of Carboniferous age. This also suggests that the Upper Member of the Pilot Shale, absent at Dutch John Mountain, may be middle to late Fammenian, Strunian, or early Carboniferous.

Acknowledgments

Thanks are due the Illinois State Academy of Science for a research grant in aid to Gordon and O'Brien. C. W. Collinson processed and identified conodonts. A. H. Coogan and V. A. M. Langenheim aided in field work. W. W. Hay, N. R. O'Brien, Karl Hornyik, and James Shea helped in the laboratory or in manuscript preparation.

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Manuscript received April 7, 1967.

HABITAT, HABITS, AND VARIATION OF KINOSTERNON SUBRUBRUM IN SOUTHERN ILLLINOIS

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ABSTRACT. — Five years of observations on two populations of *Kinosternon subrubrum* in southern Illinois reveal that the species is abundant in temporary ponds of oak flatwoods. It has been rarely collected because it burrows into the ground when the ponds dry each spring. Dominant plants in the habitat are buttonbush, swamp cottonwood, cypress, black willow, swamp white oak, and pin oak. Dimensions are given for 22 adult, 5 hatchling, and 3 juvenile mud turtles. Variation is discussed.

The eastern mud turtle, *Kinosternon s. subrubrum,* has been considered a rare reptile in southern Illinois. The only specimen Cahn (1937) had was a shell from near Cairo in Alexander County. Cagle (1942) reported three specimens, two from Williamson and one from Jackson counties; Cagle (1946) reported one specimen from near Dixon Springs in Pope County. Smith (1961) added a fifth locality in Saline County, and Klimstra and Hutchison (1965) reported it from Johnson County. We have seen two mud turtles from Freeman Spur in Williamson County, one from Sandusky in Alexander County, and several from Mermet in Massac County, where it is an abundant species. The observations reported here refer generally to mud turtles in southern Illinois but are based on two populations at Mermet, which were studied from the spring of 1962 to the spring of 1966. We feel that this turtle has been rarely collected because it inhabits mainly temporary woodland ponds. When the ponds dry up during the spring, the turtles burrow into the ground where they remain until the following year.

HABITAT

The optimum habitat for the mud turtle in southern Illinois lies in sections of the Pin Oak Flatwoods that annually accumulate enough water to allow development of bald cypress (*Taxodium distichum*), buttonbush (*Cephalanthus occidentalis*), swamp cottonwood (*Populus heterophylla*), and black willow (*Salix nigra*). Other important trees are swamp white oak (*Quercus bicolor*), and pin oak (*Quercus pulustris*). Buttonbush and cottonwood are the most frequently encountered species. Each spring these areas contain standing water up to one meter deep, but more commonly 15 to 45 cm deep. The water is clear but becomes filmed with dust and algae by late April. The bottom is hard and covered by a thick layer of leaves. The vegetational aspect is of a lower layer of buttonbush and willow thickets, which give way to medium sized cottonwoods and larger oaks. Cypress is always in the crown.

The most abundant reptile in such areas is *Kinosternon subrubrum.* Despite the leaf litter and other cover, it was often possible to find 10 adults within an estimated 7 meter square area. The only other turtles seen were two common snapping turtles (*Chelydra s. serpentina*) in 1963. The only snakes seen were several large diamondback watersnakes (*Natrix r. rhombifera*) in 1963, and a single mud snake (*Farancia abacura reinwarti*) in 1964.

We have noticed a difference in the habitat of mud turtles and musk turtles (*Sternothaerus odoratus*) throughout southeastern United States. Musk

turtles prefer deeper, more permanent bodies of water, and the two species are rarely found together. We have not seen them together in Illinois.

Habits

Spring emergence of the mud turtle takes place in mid-March (Schwegman; pers. comm.). We were able to find no turtles in late February of 1965. On March 28, we found 7 sluggish individuals, 4 males and 3 females. The water temperature was 8.8 C. The population was not yet fully active, and dates of relative activity probably vary from year to year depending on the temperature. As is typical of the species, the mud turtles of Massac County spend their time resting or foraging in shallow water. They were commonly seen in water too shallow to cover the carapace, and a few were seen on land. Many were hiding in the leaf litter below the surface and occasionally stretched to the surface for air. Basking was not observed. The only sunshine received by these turtles is that which filters through the tree branches in early spring.

The annual evaporation of water in the flatwoods forces the mud turtles to bury themselves in the ground. On April 21, 1963, the water was reduced to shallow pools surrounded by soggy ground. We first noticed a burrow when we saw a turtle trying to escape into a hole 13 cm deep. The shape and size of the opening were the same as that of the turtle in cross section. Three days later, openings to burrows were very numerous. Most were shaped like a mud turtle in cross section. Many were plugged with mud that had been pushed upward in the digging process. Some were filled with water. No burrow contained more than one turtle. Burrows ranged from 20 to 40 cm deep; deeper burrows terminated in a cavity. Activity of the turtles varied depending on what stage of desiccation their particular area had reached. In the wettest areas, individuals were at the mouth of the burrow facing outward. These tried to retreat when approached. Individuals in dryer areas were deep in the burrows but were easily pulled from them. These individuals were covered with mud and appeared to be very sluggish. They did not pull the head and limbs into the shell as mud turtles normally do.

By the end of April, the area was virtually dry, and no turtle was seen. Dry flatwoods are a most unlikely looking habitat for a population of aquatic turtles, and no evidence, save possibly a shell, indicates their presence. In 1963 the population was active for an estimated 6 weeks. The forest floor was hard and dry throughout the summer, and water did not begin to accumulate till winter. The situation was similar in the following three years, although burrowing occurred in early May rather than in April.

Drying of the flatwoods ponds in Massac County is a natural, yearly occurrence to which the mud turtles apparently are adapted. We have found no other report on this type of annual activity in turtles. Reports on the reactions of this and other species due to pond drying vary. Wetmore and Harper (1917) reported the spring emergence of *Kinosternon pennsylvanicum* (*K. subrubrum*) from a hole 46 m from a marsh in Virginia. This may not have been related to pond drying. Cahn (1937) stated that this species has been found in dry soil, but he gave no further details. Carr (1952) reported a mass migration of *K. s. hippocrepis* from a drying lake in Texas. Cagle (1944) observed snapping turtles buried in the bottom of a drying lake. Klimstra (1951) reported movements of snapping turtles, probably due to water loss in the normal habitat. Carr (1952) stated that the Florida snapping turtle (*Chelydra s. osceola*) either buries in the mud or moves when ponds dry. We have seen snapping turtles that did not burrow and that did not survive abnormally dry conditions. Some turtles (*Chrysemys, Pseudemys*) are not prone to bury in the ground as the habitat dries but wander in search of more water. We have seen hundreds of turtles (*Pseudemys* and others) in southern Florida that were unable to survive the loss of water in canals they inhabited. In the early spring of 1964, we observed 30 shells of *Sternothaerus* and 5 of *Chrysemys* that did not survive the partial drying in 1963 of the LaRue Swamp in southern Illinois. Most of the musk turtles appeared to have been killed by some predator. Three individuals, however, were found alive under a log, where they probably had been since the previous summer. Nemuras (1966) discussed temporary water bodies in Maryland which contained

TABLE 1.—Dimensions (mm) of Adult Mud Turtles, *Kinosternon subrubrum*, from Mermet, Massac County, Illinois.

Character	Specimen																				
	Males (1-7)							Females (8-22)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
CARAPACE																						
Length	104	85	85	89	90	88	99	113	88	88	98	89	94	78	83	93	93	95	88	88	95	101
Width	75	60	61	70	66	65	70	82	65	66	68	60	68	61	62	68	69	69	67	60	72	74
Height	37	38	31	36	32	34	39	42	37	40	43	40	40	33	36	38	39	37	39	33	40	42
PLASTRON																						
Length	95	77	76	87	81	78	86	103	83	81	89	76	92	75	80	86	84	84	83	80	86	91
Width	59	49	49	53	52	50	52	63	53	54	56	46	56	49	49	54	49	53	53	45	56	63
Gular Section	32	30	28	32	32	29	32	39	30	30	31	28	32	27	30	31	32	32	31	30	30	33
Abdominal Section	25	17	19	22	17	19	21	25	21	20	21	17	25	17	20	20	19	21	21	21	21	24
Anal Section	34	28	29	32	31	30	30	39	32	31	34	31	35	28	29	33	28	31	28	29	34	34
BRIDGE	17	13	14	14	15	14	16	23	17	15	18	14	16	13	14	16	13	17	16	16	19	18

bog and spotted turtles (*Clemmys*). He stated that, as the ponds dry in late summer, the turtles would "have to estivate to keep alive." Mud and snapping turtles also occurred in the area, and one of the latter was found in a hole when the pond was dry.

Variation

Approximately 75 mud turtles were collected and examined during the course of this study. The collections were made from two populations in the vicinity of Mermet. The dimensions of 22 adults collected in a single day in April of 1963 are given in Table 1. The following remarks on variation refer to these specimens and to juveniles and hatchlings collected from the same populations but on different dates.

Secondary sexual differences have been discussed by Carr (1952), Grant (1935), and by Smith (1961). The shell shape, color, and size are similar in both sexes, but the female generally has a proportionately higher carapace and a longer plastron. Old males have a moderately enlarged head and an anteriorly widened carapace in which the widest point is at the suture of the first and second laterals.

The carapace length of hatchlings varies from 20 to 25 mm (Table 2). The carapace and soft parts are black. The plastron is black at the center with a broad, irregular, red-orange to orange periphery, which fades to pale yellow during the first year. The carapace width and height are greater in proportion to its length in hatchlings (Table 2). The plastron length is proportionately shorter than the carapace making protection of the soft parts impossible in very young mud turtles.

The characteristically bright color of hatchlings quickly fades. After 60 days in captivity, the black carapace of five individuals faded to light or dark brown with traces of black remaining in two specimens. The orange coloration faded to light orange or to pale yellow. Nine months later no orange or yellow color remained.

Young mud turtles approximate the shape and color of adults during the second or third year, although several years are needed before they reach sexual maturity. The major feature that persists in juveniles is that the carapace remains widest at the posterior end rather than at the middle or the anterior end.

Most adults range from 85 to 95 mm in carapace length. The largest specimens are a male 104 mm long and a female 113 mm long. The carapace is usually flattened on top with the high-

Table 2.—First Year Growth of Five Captive Mud Turtles, *Kinosternon subrubrum*, from Mermet, Massac County, Illinois.

	Specimen				
	1	2	3	4	5
Carapace Length					
May 4, 1962	20	23	23	21	25
June 29, 1962	23	25.5	27.5	27.5	28.5
April 4, 1963	26	27	30	29	30
Carapace Width					
May 4, 1962	16	18	19.5	17	20
June 29, 1962	18.5	21	22	24	24
April 4, 1963	22	22	26	24	26
Carapace Height					
May 4, 1962	11.5	13	14	12	14.5
June 29, 1962	12	14	14	15	15
April 4, 1963	13	14	15.5	15	16.5

Table 3.—Dimensions (mm) of Three Juvenile Mud Turtles, *Kinosternon subrubrum*, from Mermet, Massac County, Illinois.

	Specimen		
	1	2	3
Carapace			
Length	59	39	27.5
Width	45	33	24
Height	25	17	13
Plastron			
Length	54	34	23
Width	38	27	17
Gular Section	20	12	8.5
Abdominal Section	13	8	6
Anal Section	21	13	8.5
Bridge	10	6	2.5

est point being at the center or at the suture of the fourth and fifth centrals. The shell is uniformly brown or olive-brown. The plastron is usually light brown to creamy tan at the margin becoming brown to dark reddish brown or black at the center. Some individuals have either a uniformly dark brown or a mostly tan plastron. Margins of the scutes are black. The head is patterned with bold yellow markings on a brown or olive-brown background. Confluence of the markings sometimes forms a line from the eye to the angle of the jaws, but distinct lines typical of *K. s. hippocrepis* are absent. Illinois specimens are similar to those we have seen from Alabama and Georgia.

Reproduction

On April 12, 1964, we found two copulating pairs of mud turtles in shallow water at Mermet. Although no nests were found, it is probable that nesting takes place in April or in early May before the drying of the ponds. It would seem necessary for the young to remain in the nest till the following spring. This is supported by the discovery in 1962 and in 1963 of five hatchlings in a roadside ditch. In both years the individuals were within three meters of each other indicating that they had recently emerged from a nest. According to Nichols (1947), spring emergence of hatchlings is the rule on Long Island, New York.

Two eggs laid in captivity by a 113 mm individual measured 30.5 by 16.5 mm and 28 by 17 mm.

Disposition

In nature, mud turtles are timid and inoffensive. As Grant (1935) observed, newly captured individuals do not attempt to bite, and there is little or no odor given off by this species as there is by certain other species of *Kinosternon* and by *Sternothaerus odoratus*. When captured, individuals attempt to hide in leaf litter, under a log, or in a burrow; when picked up, they simply withdraw into the shell. The temperament of some mud turtles changes in captivity; these become less prone to withdraw into the shell and commonly try to bite.

Acknowledgments

We are grateful to Dr. Ronald A. Brandon, Department of Zoology, Southern Illinois University, Carbondale, who read the manuscript and who offered many helpful suggestions during its preparation. Discussions with Dr. George W. Folkerts, Department of Zoology-Entomology, Auburn University, Auburn, Alabama, contributed much to this study.

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Manuscript received, April 6, 1967.

SOME MORPHOLOGICAL AND BEHAVIORAL CHARACTERISTICS OF AN INTERGRADE POPULATION OF THE GREEN FROG, *Rana clamitans*, IN SOUTHERN ILLINOIS

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ABSTRACT. — A sample of 475 green frogs, representing an intergrade population of *R. c. clamitans* x *R. c. melanota*, was collected in southwestern Illinois. Variations were found in this population as compared with findings reported for green frogs in the northern portion of the range. These differences include: (1) shorter body length at metamorphosis, (2) smaller length at maturation, (3) shorter maximum length, (4) longer breeding period, and (5) longer period of annual activity. Supporting data are offered for biannual metamorphosis. Additional morphological aspects and seasonal behavior of the green frog are reported.

The green frog, *Rana clamitans*, is an anuran whose distribution encompasses almost all of the eastern United States. Although this frog has attracted the interest of many investigators, Smith (1950, p. 103) states that nothing is known of the life history of the green frog for populations in the central and southern portions of its range. *R. c. melanota* has been the subject of significant ecological studies in New York and Michigan such as those of Hamilton (1948), Martof (1953a, 1953b, 1956a, 1956b), Munz (1920), Ryan (1953), Wright (1914), and Wright and Wright (1942); however, little work has been done on the southern subspecies, *R. c. clamitans*.

Frogs of the present study were collected from the Pine Hills area of southern Illinois, and as Rossman (1960, p. 214) has described, represent an intergrade population of *R. c. clamitans* x *R. c. melanota*. This population exhibits some divergent behavioral and morphological characteristics from those reported for more northern animals. The following data are of particular worth from a comparative standpoint, being of further value when greater attention is given to the natural history of the green frog in its southern-most range.

MATERIALS AND METHODS

A total of 475 green frogs were collected in weekly samples from the Pine Hills region of Union County, Illinois from March 1963 to February 1964. Most animals were taken from or near LaRue Swamp. A thorough floristic description of the area is given by Ashby and Kelting (1963) and Mohlenbrock (1959).

Frogs were hunted where their numbers seemed greatest. No selection was made as to size of frog collected, but there was an attempt to obtain 20 juveniles and 20 adults per month. After capture the frogs were taken to the laboratory and frozen in plastic bags for future processing. Upon thawing each frog was placed dorsum down on a millimeter rule with gentle pressure applied so the cranium and vertebral column were aligned and straight. The snout-vent length was measured from the snout to the tip of a pointer whose shaft was pressing the posterior edge of the ischium. Length was recorded to the nearest millimeter. Determination of the tympanic diameter was made along the longitudinal axis between the outer perimeter of the left tympanic ring; the resulting value was recorded

to the nearest half millimeter. Other seasonal data were gathered from the sample, including stomach contents (Jenssen and Klimstra, 1966), organ weights, and an endoparasite examination.

Results and Discussion

Metamorphosis

The size of transforming green frogs from southwestern Illinois appears smaller than those found further north. Snout-vent lengths of 15 juvenile frogs from Pine Hills which still possessed tail stumps ranged from 23 to 33 mm with a mean length of 27.2 mm; this value agrees well with the 26.1 mm average obtained from 34 transforming frogs from adjacent Jackson County. Some of the latter specimens were utilized in a dietary study of larval and transforming green frogs (Jenssen, 1967).

From Ann Arbor, Michigan, Martof (1956a, p. 106) reports the snout-vent lengths of newly transformed frogs, those possessing tail stumps, as ranging from 28.4 to 36.3 mm with a mean of 32.6 mm. Data collected in Ithaca, New York by Munz (1920, p. 41) showed a range of 29 to 36 mm for 15 transforming green frogs (tail stumps 2 to 15 mm long) and a mean of 32.5 mm; this is almost identical with Martof's observations. Both investigators report significantly larger snout-vent lengths than those measurements taken of frogs in southwestern Illinois.

Traditionally, the green frog tadpole was thought to overwinter before completing its larval development. However, the recent works of Martof (1956a, 1956b) in Michigan, Richmond (1964) in Pennsylvania, and Ting (1951) have produced strong evidence that development from egg to frog can occur within the same season. A similar conclusion is drawn for the Pine Hills population.

Evidence for a biannual metamorphosis is indicated in Figure 1. The length frequencies for the fall-

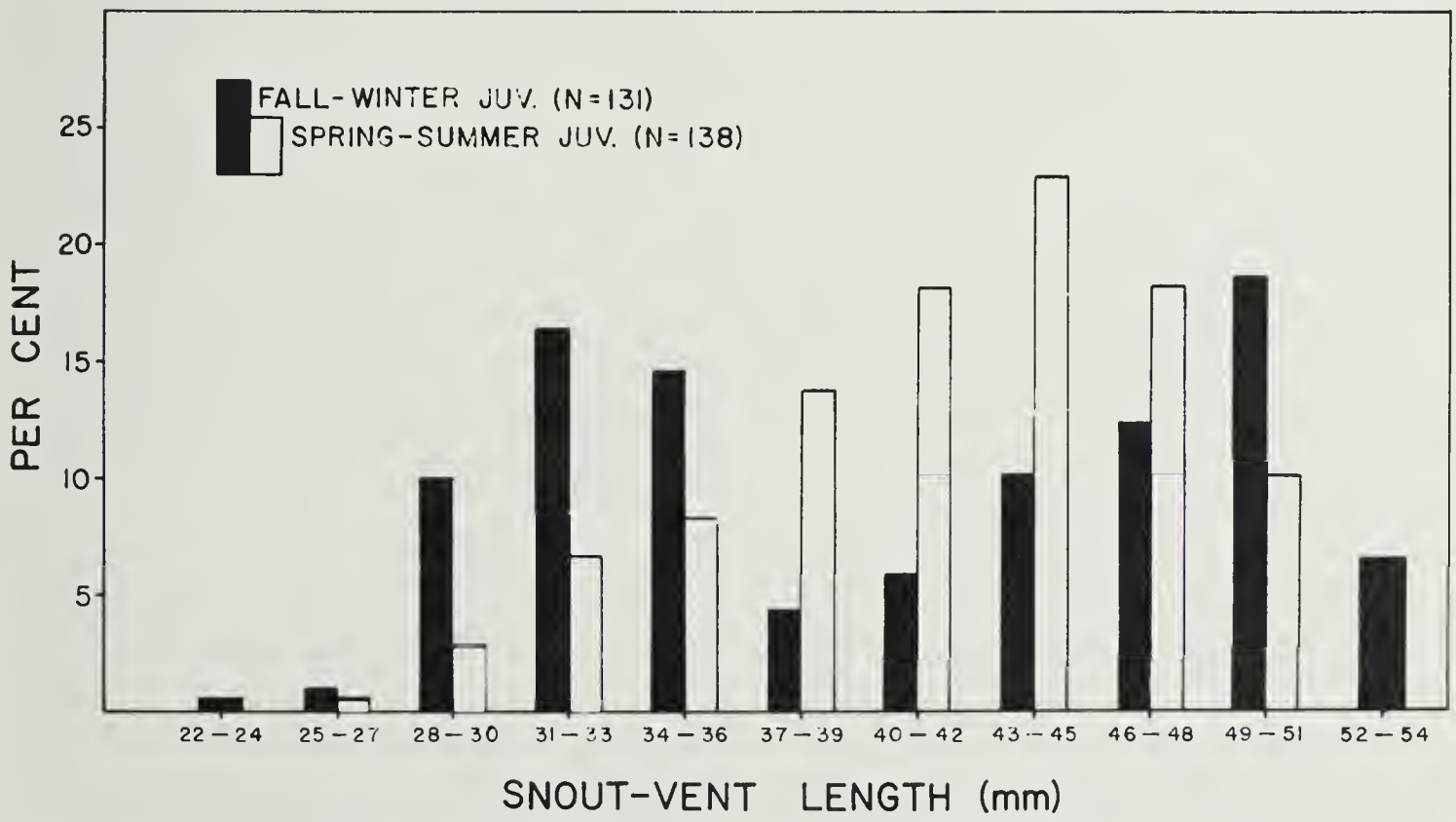

Figure 1. — The percent frequency of occurrence of snout-vent lengths of juvenile green frogs collected Mar.-Aug., 1963 (Spring-Summer) and Sept., 1963—Feb., 1964 (Fall-Winter), LaRue Swamp, Pine Hills, Union County, Illinois.

winter juveniles illustrate a distinct bimodal distribution. The first peak includes approximately 30% of the sample within snout-vent lengths of 31 to 36 mm. In all probability these frogs represent the late summer transformation. Many of the larger animals are products of the acknowledged early summer metamorphosis.

Furthermore, collections and observations of small juveniles with tail remnants were made only during May 24 through June 29 and August 28 through September 5. Field notes record the largest numbers of newly transformed frogs were seen on June 12 and September 1. The period of late summer transformation is probably greater than the dates given as no trips to the study area were made between September 5 and September 23. In comparison with the Illinois dates, Martof (1956a, p. 105) found metamorphosis near Ann Arbor to occur during June 5 through July 12 and August 3 through September 28.

Juveniles

The snout-vent lengths of juveniles ranged from 23 to 54 mm. There was no consistent body length which delimited juveniles from adults (Figs. 1 and 2). Besides the natural hereditary variations involved in maturation, sex of the frog and season had a large influence on the size at which adulthood was reached.

The largest non-adults were females. Of the juvenile frogs having body lengths between 49 and 54 mm, 30 were females as opposed to 16 males. No juvenile males were found in the 52-54 mm class. Martof (1956a, p. 113) reports males maturing before females, but could find no significant difference in growth rate for juvenile males and females. He concluded it took longer for gametic development in females than males.

The largest juveniles were found in the fall-winter samples. Subadults passing through the breeding season without attaining sexual maturity would possess adult lengths by fall. There is little confusion in classifying males as there are obvious secondary sexual characteristics which appear at puberty, such as enlarging tympanums. However, the author knows of no adequate means of ascertaining the age category of a female frog when it is between 49 and 55 mm long without consideration of the season and an internal examination.

Adults

Referring to Illinois green frogs, Smith (1961, p. 97) states that adult females are said to be larger than adult males, but the size difference was not obvious from the series of frogs available. Although Ryan (1953, p. 77) reports a greater size for adult females in his New York study, Martof (1956a, p. 113) could find no difference in mean lengths of the sexes taken from 344 adult males and 307 adult females from Michigan.

The length frequencies of 206 Pine Hills adult frogs are presented in Figure 2. A distinct sexual difference in length is obvious. It is felt that a representative sample of large males was obtained since during the three month breeding season

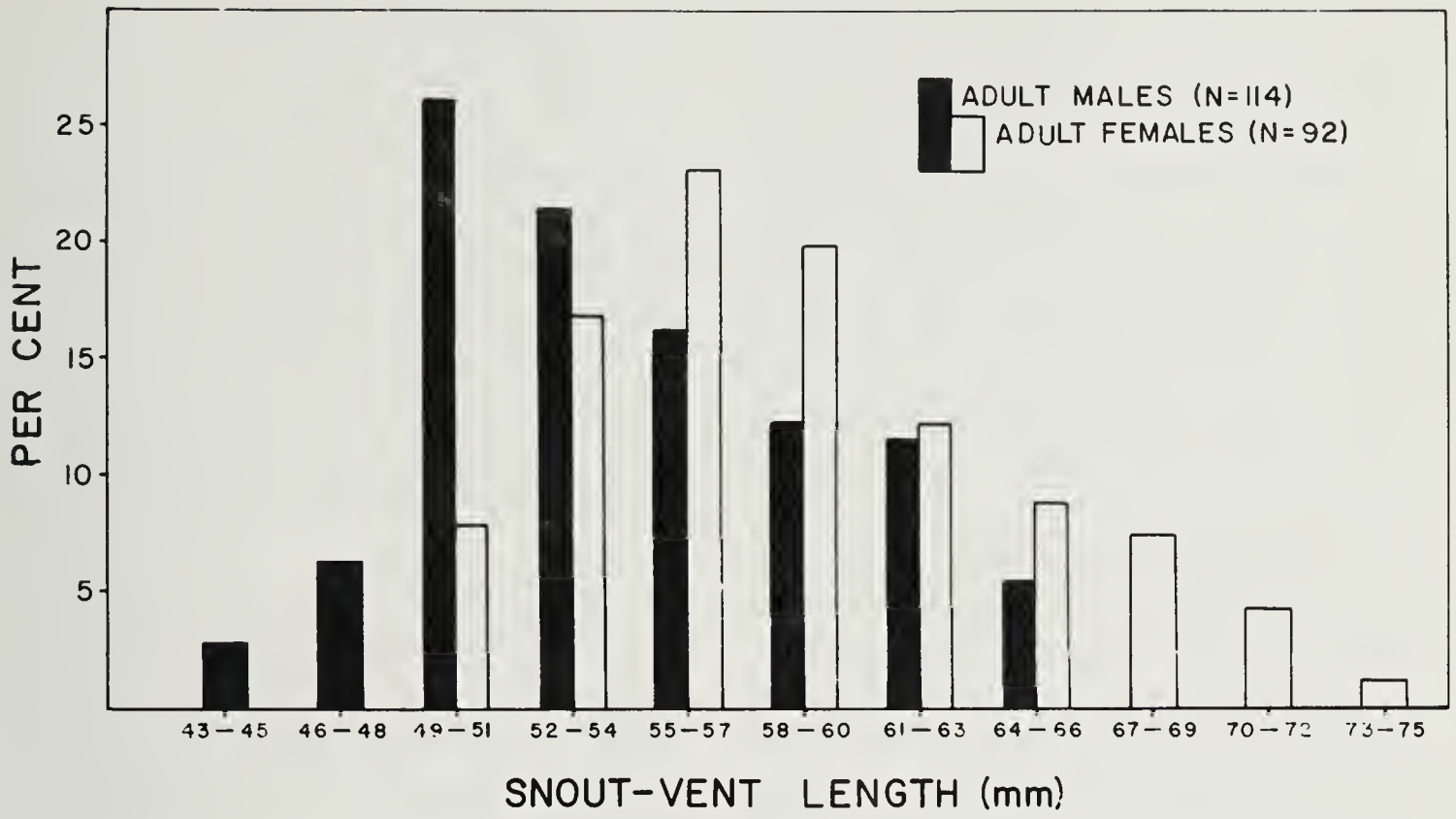

FIGURE 2. — The percent frequency of occurrence of snout-vent lengths of adult green frogs collected Mar., 1963—Feb., 1964, LaRue Swamp, Pine Hills, Union County, Illinois.

the calling led the collector to the adult males. Still the largest male (66mm) is considerably smaller than the largest female (75mm). Adult male and female mean snout-vent lengths are 54.6 mm and 59.0 mm, respectively. The smaller adults were collected in late spring and summer, the periods when subadults come into sexual maturity.

In comparing the above values with lengths recorded by other workers, Martof (1956a, p. 112) found adult males in Michigan matured at 60-65 mm, had a mean length of 79.8 mm, and reached a maximum size of 103 mm. The adult females matured at 65-75 mm, had a mean length of 80.3 mm, and reached 105 mm in size. Wright and Wright (1942, p. 192) state green frogs in the south (presumably *R. c. clamitans*) are smaller than northern frogs. They give adult male snout-vent lengths as ranging from 52 to 72 mm and the adult females 58 to 75 mm; these values agree closely with lengths found in the Pine Hills population, though some of the latter frogs exhibit maturation at a smaller size.

Martof (1956a) presents a reliable field technique for recognizing adult males. By dividing the snout-vent length by the diameter of the tympanum, one derives the sex index. Martof (1956a, p. 115) found that if the index was less than 10, the frog from which the measurements were taken was an adult male. Individuals with an index of 10 or greater were classified as either juveniles or adult females.

This sex index was applied to the intergrade population of Pine Hills and was found to be accurate. How-

ever, in these frogs the dividing point for adult males was 9 or less with juveniles and adult females being greater than 9 (Fig. 3). The only overlap occurred in the 9.0-9.4 class; here existed a number of subadult males approaching maturity while some of the remaining frogs were just acquiring adulthood as evidenced by enlarging gonads and thumbs.

It is not too surprising that a comparison of the sex index of Michigan frogs with that of the Illinois frogs should reveal a difference since there are other morphological variations between the two populations. The disparity in the allometric relationship of the body length to tympanum size found between the frogs of the two states may possibly be related to the smaller snout-vent length of the southern subspecies. In computing the sex index, it can be seen that a decreased body size divided by a constant tympanic diameter results in a smaller index value.

The sex index is of particular use when working in the field with *R. c. clamitans,* as many adult males of this subspecies do not have a yellow throat and the enlarged thumb is a character of subjective evaluation. The snout-vent/tympanum index, however, can be applied precisely throughout the year.

Seasonal Activity

In November juvenile frogs remained common along the swamp shoreline long after adults disappeared. Juveniles were collected in the swamp proper up to November 26. In contrast, the last adults were taken from the swamp on November 9. Near Ann Arbor, Michigan all frogs were hibernating between October 29 and November 13 (Martof, 1956b, p. 225). Throughout the winter many Pine Hills juvenile frogs

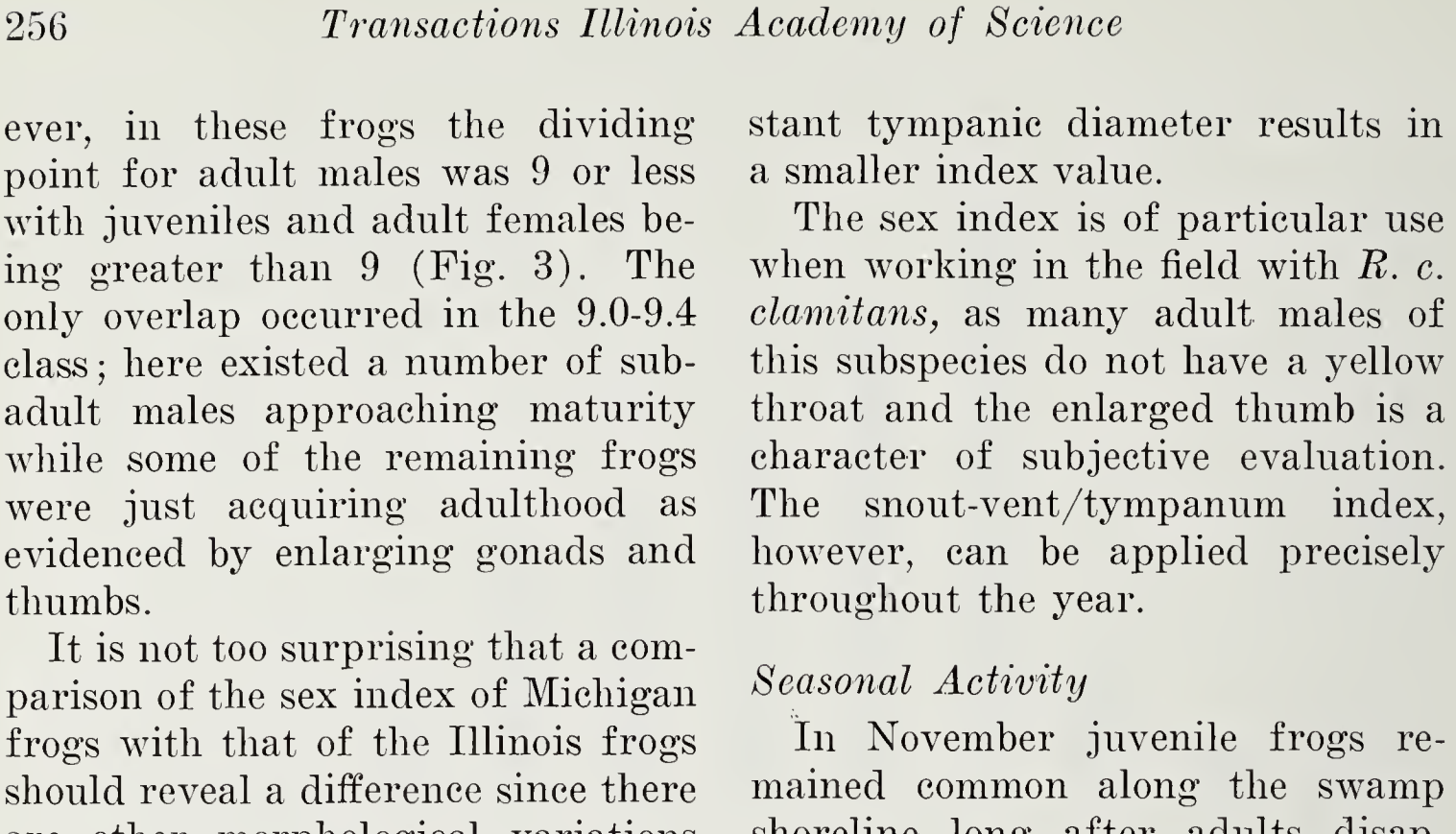

FIGURE 3. — The percent frequency of occurrence of the sex index ratio (snout-vent length/tympanic diameter) of green frogs collected Mar., 1963—Feb., 1964, LaRue Swamp, Pine Hills, Union County, Illinois.

remained active in the small spring-fed streams which run into the swamp from the base of the adjacent limestone bluffs. These frogs were frequently captured away from any apparent hibernaculum. Presumably, the young frogs were foraging as evidenced by a smaller percentage of empty stomachs among juvenile frogs than the adults during much of the winter (Jenssen and Klimstra, 1966, p. 171).

Adults also overwintered in the streams, but were seldom judged to be active as most were found confined within small chambers beneath rocks, mud banks, and leaf debris. Many adults were discovered under the snow in the cavities of rock piles where seepage water from the bluffs trickled through their hibernacula; these microhabitats were never found frozen even though the nearby swamp was covered with 3 to 4 inches of ice. On one occasion a gathering of 5 large green frogs was seen through the swamp ice. A culvert carried spring water into the area and the animals were probably responding to the increased water temperature.

Other investigators have reported overwintering behavior of the green frog. During a warm rain on December 18 in New York, Sanwals (1916) observed an active adult near a pond. Martof (1956b, p. 225) uncovered hibernating frogs during early spring buried in the bottom mud of a stream. A detailed description of the climatic factors and microhabitat of a hibernaculum in Michigan is given by Bohnsack (1951). And Pope (1947) has found green frogs to be active about springs in the Chicago area during mild winter temperatures.

Frogs from Pine Hills began emerging from hibernation during the second week in March. After a series of rains around March 22, frogs were prevalent especially in the flooded woodland along the swamp. As in November when juveniles were the last to go into hibernation, it appeared the juveniles were the first to emerge as they were abundant while adults were at first scarce. Northern green frogs appear approximately two weeks later than those in southern Illinois. Near Ithaca, New York, Ryan (1953, p. 76) placed emergence during the last week of March or the first week of April. Martof (1956b, p. 224) also found Ann Arbor frogs appeared during the last week of March. However, emergence can be as deferred as late April in southern Wisconsin (Breckenridge, 1944, p. 79).

The onset of the breeding period is heralded by the first calls of the males. In the present study calling was first heard on April 20; it continued until August 29, with no calls heard from September 1 on. Peak choruses were estimated as occurring between June 3 and August 18. The breeding period as reported by other authors is relatively consistent over the green frog's range, yet there is a trend for a shorter breeding period with an increase in latitude (Table 1). Because there can be some disagreement concerning what constitutes the breeding period (ie. calling period versus the period of

egg laying), Table 1 lists the authors' specific designations.

Summary

A total of 475 green frogs were collected in weekly samples throughout a year, March 1963 — February 1964, in southwestern Illinois. The collections represented an intergrade population of *R. c. clamitans* x *R. c. melanota*. Some variations were found in this population as compared with findings reported for green frogs in other portions of their range.

1. The mean snout-vent length of newly transformed frogs from southwestern Illinois was smaller (26.5 mm) than the mean lengths reported for northern green frogs (32.6 mm).

2. Field observations and seasonal body length frequencies indicate a biannual transformation, one in early summer and the second in late summer-early fall.

3. Females were the largest juvenile frogs taken during most months, suggesting that males may mature at a slightly earlier age.

4. Seasonal snout-vent length frequencies showed the largest juvenile frogs were captured in the fall and winter; many were of adult proportions.

5. Frogs of the Pine Hills adult population are smaller than northern green frogs. Males ranged between 44 and 66 mm with a mean of 54.6 mm. Females showed a greater size than males, ranging between 49 and 75 mm with a mean of 59.0 mm.

6. To aid the field investigator's ability to distinguish between a precociously matured male and a large subadult, Martof's sex index (snout-vent length/tympanum diameter) was applied and found accurate. Adult males possessed an index value of less than 9 while for adult females and juveniles it was greater than 9; however, the corresponding value for Michigan frogs was 10.

7. Green frogs in southwestern Illinois have a longer yearly activity period than frogs of Wisconsin, Michigan, and New York. Hibernation at Pine Hills began in early November for adults and late November for juveniles and lasted til early March. Spring-fed streams in the collecting area permitted some frogs to forage intermittently throughout the winter.

8. Calling was first heard at Pine Hills on April 20 and lasted til

Table 1.—The breeding period of the green frog, *Rana clamitans*, as reported across its range.

Locality	Dates of Calling	Dates of Breeding Season	Authority
New York		Late May—2nd Wk. August.	Ryan (1953)
New York		Late May—Mid August	Wright (1942)
Michigan	May 5—Mid August	Late May—Mid August	Martof (1956a)
Illinois	May—September		Smith (1961)
Georgia	April 28—September 5		Wright (1932)
Florida		April 11—August 15	Carr (1940)

August 29. Peak choruses seemed to occur between June 3 and August 18. The calling period at Pine Hills is longer than in the northern portion of the green frog's range and shorter than in the southern part.

Acknowledgments

Dr. W. D. Klimstra is gratefully acknowledged for making this study possible; it is a contribution from Project No. 10, Cooperative Wildlife Research Laboratory, Southern Illinois University. Present address of Jenssen is, Dept. of Zoology, University of Oklahoma, Norman, Okla.

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Manuscript received June 10, 1967.

COMPARATIVE DEPRESSION OF GROWTH IN MICE BY DIETHYLSTILBESTROL, ESTRADIOL AND ESTRONE

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ABSTRACT. — High estrogen concentrations in the diets of mice greatly inhibit their growth. The synthetic estrogen, diethylstilbestrol, is 5-10 times more potent than either natural estrogen (estradiol or estrone) in this respect and may act chiefly by reducing food intake. Testis and seminal vesicle weights also are more effectively reduced by diethylstilbestrol than by the natural estrogens.

Dietary estrogen administration is a convenient method of inducing mammary tumors in mice. High concentrations of a synthetic estrogen, diethylstilbestrol (DES), however, have been noted to inhibit growth and cause subsequent death of experimental animals due to inanition (Gass *et al.*, 1964). Inanition *per se*, as well as general debilitation of experimental animals, tends to reduce the frequency with which tumors develop — thus complicating quantitative studies on tumorigenesis (Tannenbaum, 1959).

Several authors (Richards and Kueter, 1941; Meites, 1949; Hartsook and Magruder, 1957) have reported that injection of both natural and synthetic estrogens inhibits body-weight gain in rats. The effects of estrogens on weight gains in rodents, then, are opposite those observed in beef cattle (where DES has been widely used as a "growth stimulant").

The present investigation was undertaken to compare the degree of growth depression in mice elicited by oral administration of the natural estrogens, estradiol and estrone, with that produced by the synthetic, DES.

MATERIALS AND METHODS

Estradiol (as estradiol-17β) was obtained from the California Corp. for Biochemical Research, Los Angeles; estrone from Nutritional Biochemicals Corp., Cleveland, O.; DES (α, α'-diethyl-4, 4'-stilbenediol) from Matheson, Coleman & Bell, Cincinnati, O.

One-hundred male Swiss mice (Cumberland View Farms, Clinton, Tenn.), 25 days old at the start of the diet, were randomly assorted into 10 groups of 10 mice each; 9 groups received estrogens admixed to Purina Laboratory Chow as shown in Table 1. The concentrations were selected on the basis of preliminary experiments which indicated that DES was approximately 5 times as effective as estradiol and 10 times as active as estrone in reducing body-weight gains. The control group received Chow alone. Food and water were available *ad libitum* throughout the experimental dietary period of 4 weeks, following which the mice were killed with chloroform, the testes and seminal vesicles removed, cleaned and weighed. (Seminal vesicles were severed near the junction with the urethra; fluid contents were expressed before weighing.) Body weight was measured in the early afternoon when mice are in a relative post-absorptive state since most feeding occurs at night (Anliker and Mayer, 1956).

RESULTS

The effects of estrogens on growth are summarized in Table 1. No group of mice receiving estrogen was able to approach the body weight gains made by the control group. A rep-

TABLE 1.—Effects of Estrogens on Body Weight of Maturing Male Swiss Mice.

Diet—ppb	Body Weight in Grams					Grams Gained in 4 Weeks	Gain as % of Original Weight	Grams of Food Consumed/ Day/Mouse
	Initial	1 Week	2 Weeks	3 Weeks	Final			
Control	16.2[1] ±0.75	23.7	25.0	27.1	30.0[1] ±0.84	13.8	85.2	4.6
DES 1,000	17.1 ±0.93	21.7	23.1	23.7	24.7 ±1.17	7.6	44.4	2.7
DES 3,000	16.6 ±1.06	19.2	21.1	22.5	23.1 ±0.77	6.5	39.2	3.1
DES 5,000	15.6 ±0.70	17.5	19.0	20.8	20.6 ±1.01	5.0	32.1	3.1
Estradiol 5,000	16.7 ±0.95	20.3	21.9	24.6	25.4 ±0.71	8.7	52.1	3.4
Estradiol 15,000	16.1 ±1.11	18.3	20.3	22.3	22.5 ±0.85	6.4	39.8	3.5
Estradiol 25,000	16.8 ±1.01	17.7	19.3	21.2	21.7 ±0.92	4.9	29.2	3.5
Estrone 10,000	15.8 ±0.84	21.2	22.0	25.3	25.7 ±0.86	9.9	62.7	4.2
Estrone 30,000	16.3 ±1.40	18.8	20.5	23.5	23.9 ±1.02	7.6	46.6	3.8
Estrone 50,000	16.1 ±0.68	18.2	18.8	20.0	20.7 ±0.72	4.6	28.6	3.4

All Values Are an Average of Ten Animals
[1]Mean ± Standard Error

resentative growth curve for mice receiving DES (Figure 1) reveals that depression of body-weight gain occurs in the first week of estrogen administration; this also is true of the natural estrogens at the dose levels employed in this study. After 4 weeks of estrogen treatment, growth of all experimental groups reached a plateau while the control animals were still making rapid gains. The greater relative potency of DES in decreasing body weight is apparent at each of the 3 dose levels. The degree of growth inhibition is a linear function of the concentration of estrogen in the diet over the ranges tested (Figure 2).

One-thousand parts per billion of DES in the diet were sufficient to reduce testis weight to about half the control value, whereas this effect was not equaled by 15,000 ppb of estradiol nor 30,000 ppb of estrone (Table 2). (1000 ppb in the diet are equivalent to an intake of approximately 3.5μg/day, assuming that mice consume 3.5 g of Chow per day.)

Seminal vesicles of all groups receiving estrogens are significantly smaller ($p<0.01$) than the control value (Table 2); this indicates that androgen secretion by the testes is greatly diminished or even abolished. Each dose level of DES produced the maximum observable depression of seminal vesicle development, as did the two highest concentrations of estradiol; no dose level of estrone achieved this depression.

Discussion

Several mechanisms by which estrogens might depress growth in ro-

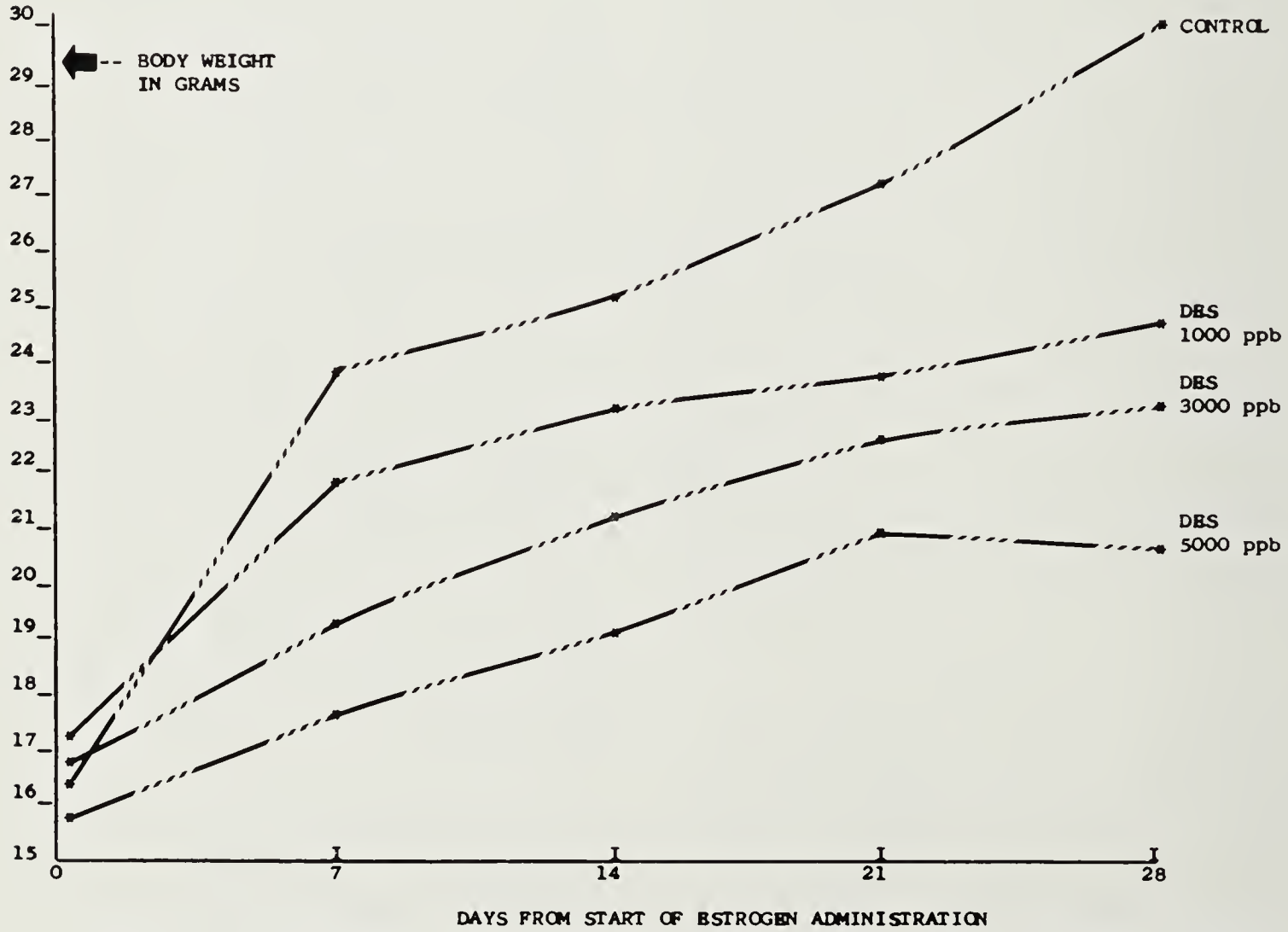

Figure 1. — Depressed Growth Rate of Male Swiss Mice Receiving Diethylstilbestrol in the Diet. (ppb = parts per billion of DES in the diet)

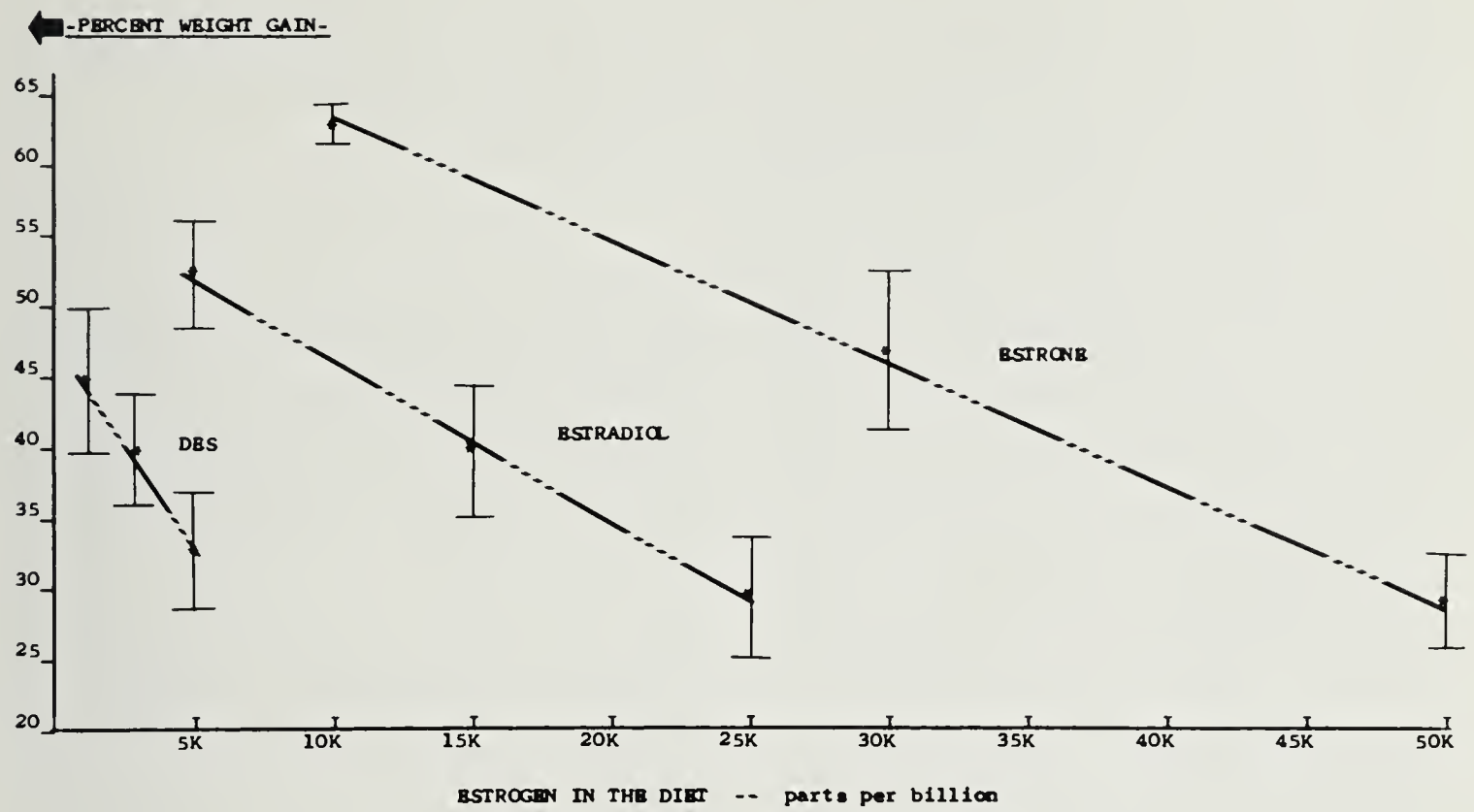

FIGURE 2. — Linear Dose-Response Relationship Between the Concentration of Estrogen in the Diet and Weight Gain in Male Swiss Mice. (Brackets indicate the standard errors of the plotted mean values.)

TABLE 2.—Effects of Estrogens on Testes and Seminal Vesicles of Maturing Male Swiss Mice.

Diet—ppb		Testes Weight in mg[1]	Seminal Vesicle Weight in mg[1]
Control		205.0[2] ±13.1	42.3[2] ±2.68
DES	1,000	105.8 ±11.2	8.2 ±0.93
DES	3,000	67.6 ± 7.7	9.3 ±0.61
DES	5,000	59.5 ± 5.1	9.1 ±0.64
Estradiol	5,000	160.0 ± 6.5	23.1 ±3.02
Estradiol	15,000	115.3 ±15.0	9.6 ±1.01
Estradiol	25,000	74.7 ± 8.0	9.0 ±1.06
Estrone	10,000	153.7 ±16.1	23.9 ±6.12
Estrone	30,000	145.8 ±20.3	21.8 ±5.56
Estrone	50,000	73.0 ± 5.9	11.5 ±1.12

All Values Are an Average of Ten Animals
[1]Paired Organ Weights
[1]Mean ± Standard Error

dents have been suggested; chief among these are that estrogens reduce anterior pituitary secretion of growth hormone (Richards and Kueter, 1941) or that they reduce food intake (Meites, 1949). Meites states that DES curtails growth in rats principally by decreasing appetite, while natural estrogens inhibit growth without a corresponding decrease in appetite. Measurements of food consumption of mice in this study show a marked decrease below control group intake at all dose levels of DES. Mice receiving natural estrogens exhibited a less pronounced restriction of appetite despite the much higher concentrations administered. Rats and mice seem to be similar, then, in regard to food intake when given either natural or synthetic estrogens. However, rats on natural estrogens (0.1mg/day) showed no reduction in growth rate until after 30 days (Meites, 1949) while restricted growth in mice occurs in the first week of estrogen treatment.

This investigation may provide a guideline to levels of natural and synthetic estrogens in the diet which allow some degree of growth in experimental mice, particularly those used in quantitative studies on carcinogenesis.

Acknowledgments

This work was reported in part at the 49th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey.

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Manuscript received, Aug. 21, 1967.

THE SHOOT APICES OF *CERCIS CANADENSIS* L.

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ABSTRACT. — The vegetative shoot of *Cercis canadensis* terminates in a low dome shaped apex with an average diameter of 80μ and an average height of 21μ. The apical promeristem organization corresponds to Popham's Type VII zonation, the Usual Angiosperm Type, consisting of a mantle, central mother cell zone, rib meristem and peripheral zone. The tunica consists of a single layer of cells. The seedling shoot apex does not differ markedly from that of the mature tree. The initiation of a leaf primordium is first indicated by periclinal divisions in the second cell layer. Axillary buds are initiated adaxial to the second or third youngest leaf primordium. There are no differences in the shape, size, or organization of the shoot apex which occurs in the axillary leaf bud and the shoot apex which occurs at the tip of an actively growing shoot. The floral promeristem, before initiation of floral parts, does not differ in shape, size, or organization from the vegetative shoot apex. The initiation of the first floral parts is accompanied by marked changes in the shape, size, and organization of the floral apex.

Since the publication of the first description of an angiosperm shoot apex in 1852, a great deal of research concerned with the shoot apex has been conducted; however, many areas remain relatively unexplored as for example very few survey studies have been attempted in a single family. There have been several comprehensive reviews of the various interpretations of the structure and activity of the shoot apex (Foster, 1939; Popham, 1951; Gifford, 1954; Wardlaw, 1957; Clowes, 1961; Romberger, 1963; Esau, 1965). Few species of the economically important Leguminosae have been studied. This investigation is concerned with the seedling and mature tree shoot apex, buds, and early leaf and flower development of the Redbud or Judas Tree, *Cercis canadensis* L.

In this paper "(vegetative) shoot apex" refers to the actively growing region at the tip of the stem and "axillary bud apex" refers to the meristematic region enclosed within the bud scales of axillary leaf buds.

MATERIALS AND METHODS

Vegetative shoot apices, axillary buds, and flower buds were collected between June 27, 1962 and July 23, 1963 from a single specimen of *Cercis canadensis* L. growing on the campus of Western Illinois University, Macomb, Illinois. Randolph's Modified Navashin Fluid (Johansen, 1940, p. 45) was used as the killing and fixing fluid. The material was transferred, without washing, directly to 70% ethyl alcohol. Dehydration and clearing were completed using a graded series of water, ethyl and tertiary butyl alcohol solutions (Johansen, 1940, p. 130). Serial sections, cut at 5μ, were stained by using a stock solution of Heidenhain's Iron Hematoxylin (Sass, 1958, p. 58) for 20 minutes and counterstaining for 20 seconds with a 0.2% alcoholic solution of fast green.

Seeds collected from the same tree in August, 1962 were forced to germinate in May and June, 1964 according to the method described by Afanasiev (1944). The vegetative shoot apex was collected from seedlings which had two or three expanded leaves. These apices were processed by the same procedure used for the material taken from the mature tree.

Observations and Results

The Vegetative Shoot Apex and Leaf Initiation. The vegetative shoot terminates in a low dome shaped apex. As measured from median or near median longitudinal sections above the level of the youngest leaf primordium, the apex diameter varies from about 45 to 105μ with the average being 80μ, and the height varies from about 4 to 44μ with the average being 21μ. The size of the apex increases during the plastochron, attaining maximum size just before the initiation of the next leaf primordium.

The organization of the apex may be adequately described in terms of the Tunica-Corpus Theory with a superimposed zonal structure. The tunica is composed of a single layer of cells which divide only anticlinally. The second and occasionally the third layer of cells could easily be mistaken for tunica layers but periclinal divisions are a regular feature its smaller, denser, less vacuolated cells arranged in four to five rows of these layers (Fig. 1). The term "mantle" is used to include the two or three surface layers in which some periclinal divisions occur. The corpus exhibits four zones: (1) the stratified cell layer(s) below the tunica, (2) a central mother cell zone, (3) a rib meristem, and (4) a peripheral zone (Figs. 1, 2). These zones are distinguishable on the basis of: (1) planes of cell divisions, (2) degree of vacuolation, (3) size of cells, (4) relative thickness of cell walls, and (5) density of the protoplasm as demonstrated by differential staining.

The central mother cell zone, composed of large, light staining, irregularly arranged cells, is centrally located beneath the mantle, usually in the third and fourth layers of cells from the summit. Below the central mother cell zone is a rib meristem consisting of four to six vertical columns of highly vacuolated cells. The peripheral zone, lateral to the cen-

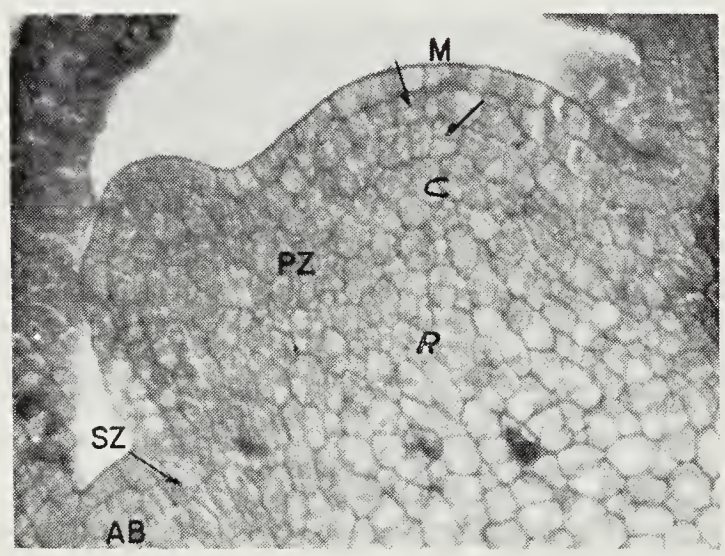

Figure 1. — Longisection of a vegetative shoot apex of *Cercis canadensis* collected in June, illustrating zonation, periclinal divisions in layers 2 and 3 of the mantle, and the shell zone of early bud development. C — central mother cell zone; PZ—peripheral zone; R—rib meristem; M—mantle; SZ—shell zone; AB—axillary bud; arrows indicate periclinal divisions. X600.

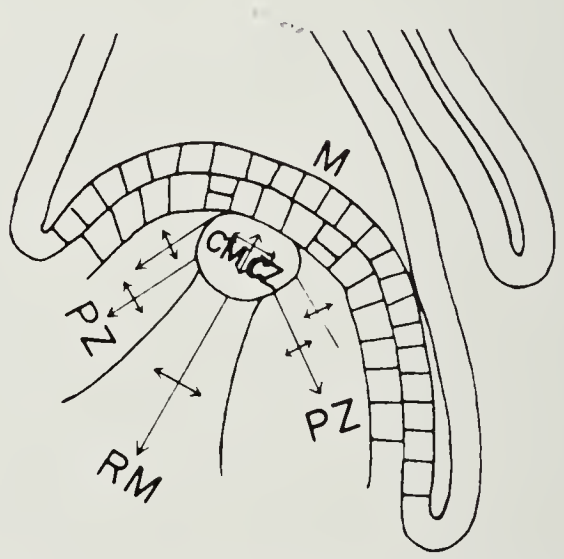

Figure 2. — Diagrammatic representation of a median longitudinal section of a shoot apex illustrating zonation. M—mantle; CMCZ — central mother cell zone; PZ—peripheral zone; RM—rib meristem. Arrows indicate planes of cell division.

tral mother cell zone, is identified by which are more or less parallel to the tunica. During leaf initiation the general uniform cell arrangement of the peripheral zone becomes disorganized on the side of the apex where initiation occurs (Fig. 3). No cambium-like zone was observed in any of the apices studied.

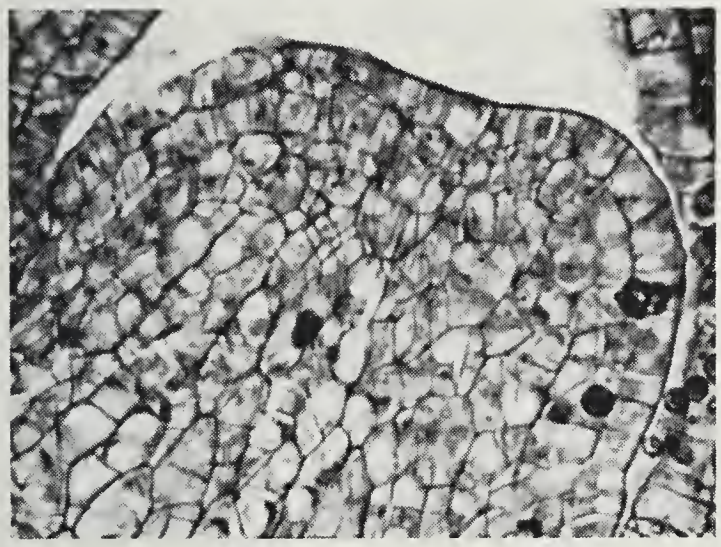

Figure 3. — Longisection of a vegetative shoot apex at minimum size showing the disruption of the peripheral zone on the side of the apex where leaf primordium initiation occurs. X1,000.

The most conspicuous characteristic of the rib meristem and the peripheral zone is their stratified appearance, the result of repeated anticlinal cell divisions (perpendicular to the surface). Occasional periclinal cell divisions (parallel to the surface) increase the number of rows as the distance from the summit increases. There is no sharp line of demarcation between these two zones. In tracing the rows of cells of the rib meristem and peripheral zone toward the summit, it is found that they converge at the central mother cell zone in which cell divisions in various planes result in no definite pattern of cell arrangement.

Mitotic figures were rarely seen; therefore, no conclusions can be drawn concerning the relative mitotic activity of the various zones. Since the tunica layer never divides periclinally and is continuous over the surface of the primordium, it presumably gives rise to only the epidermal system of the shoot. The rest of the shoot must, therefore, arise from the corpus. The position of the central mother cell zone relative to the rib meristem and peripheral zone indicates that it is probably an initial zone from which the rib meristem and peripheral zone arise. The rib meristem appears to give rise to the pith; the foliar organs, vascular tissue, and cortex of the stem being derived from the peripheral zone.

When the last formed leaf primordium has attained a height of about 90μ, the next primordium is initiated on the opposite flank of the apex. The first indication of leaf initiation is the occurrence of periclinal divisions in the outer layer of the corpus (the second cell layer) (Fig. 4). Additional periclinal divisions accompanied by cell elongation parallel to the shoot axis forms a protuberance

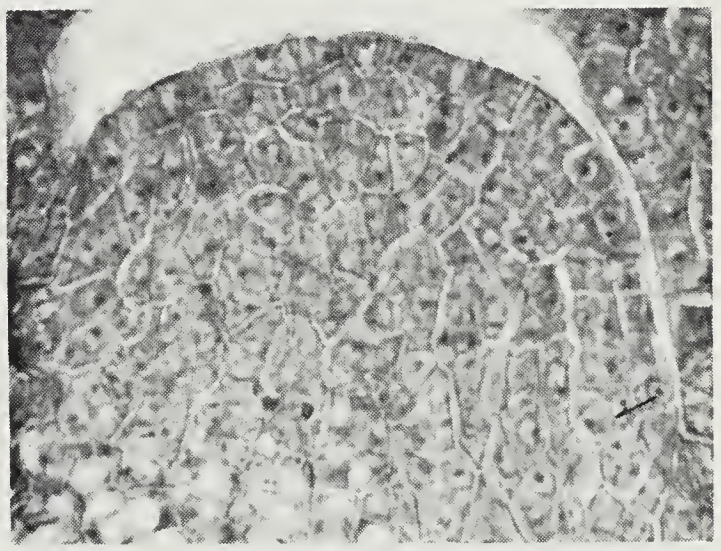

Figure 4. — Longisection of a vegetative shoot apex at maximum size showing periclinal divisions (arrow) in the outer corpus layer of the right flank of the apex. These divisions indicate the initiation of a leaf primordium. X1,000.

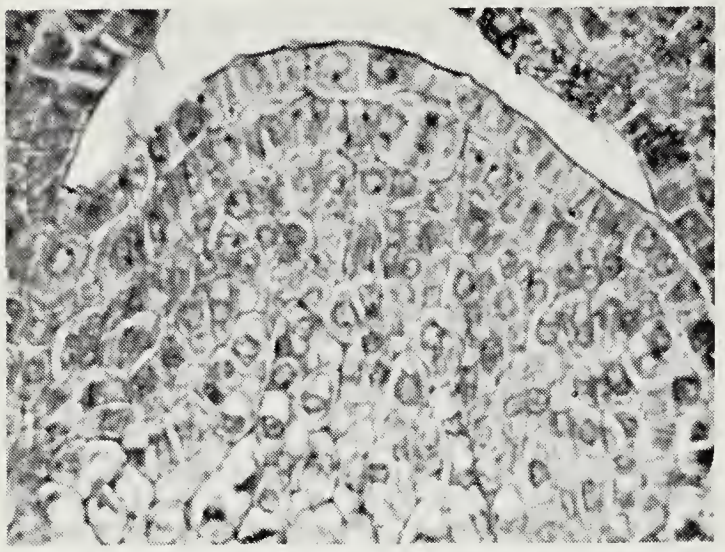

FIGURE 5. — Longisection of a vegetative shoot apex illustrating the protuberance resulting from periclinal divisions and cell elongation in a very early stage of a leaf primordium. Note also the narrow cells resulting from anticlinal divisions and the enlarged cells of the tunica on the right flank. X1,000.

on the side of the apex (Fig. 5). Further divisions in various planes derange the stratified appearance of the leaf primordium and the peripheral zone on that side of the shoot apex (Fig. 3). Growth in length of the very young primordium continues primarily by anticlinal divisions of the corpus derivatives. The adaxial surface of the leaf primordium is delimited from the lateral surface of the shoot apex by several rows of elongated cells formed as a result of periclinal divisions in the peripheral zone. The characteristic zonation of the peripheral zone is reestablished as the primordium is delimited. The tunica layer, as a result of anticlinal divisions and cell enlargement, retains its identity as a discrete layer throughout leaf initiation and subsequent leaf development (Fig. 5). Further development of the leaf has not been investigated. At the onset of leaf initiation the shoot apex has reached its maximum size (Fig. 4), but by the time the peripheral zone has become disorganized, the apex is at its minimum size (Fig. 3).

The procambium of the leaf primordia is continuous with the stem procambium. Differentiation of the procambium appears to be acropetal into the leaf from the stem procambium since isolated procambial strands in the leaf primordia were not observed.

A preliminary study of the vegetative shoot apex of *Cercis canadensis* seedlings indicated that the seedling apex does not differ markedly from the vegetative shoot apices of the mature tree. Like the apices of the mature tree, the seedling apex is dome shaped but is somewhat larger since diameters varying from 60 to 126μ and heights from 7 to 60μ were recorded when measured from median or near median longitudinal sections above the youngest leaf primordium. The seedling shoot apex size varies during each plastochron.

The tunica is composed of a single layer of cells, since, as in the shoot apex of the mature tree, periclinal divisions occur in the subsurface layers. The zonation of the corpus of the seedling shoot apex (Fig. 6) is

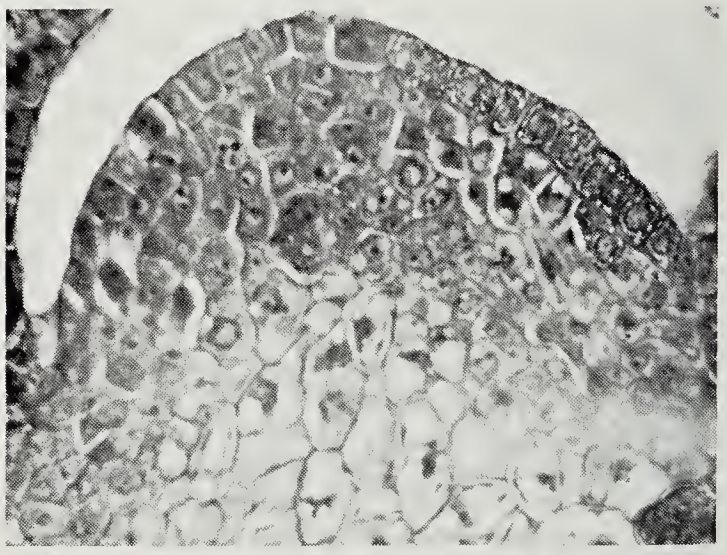

FIGURE 6. — Longisection of a vegetative shoot apex of a seedling illustrating zonation of the apex. X1,000.

the same as in the mature tree. Leaf initiation and procambium differentiation does not differ from that in the mature tree.

The Promeristem of Axillary Leaf Buds. The axillary buds of *Cercis canadensis* are usually first discernible in the axil of the third youngest leaf primordium. Occasionally bud initiation may be detected during late plastochron in the axil of the second youngest leaf primordium. An early indication of axillary bud initiation is the formation of two to four curved rows of elongated cells adaxial to the leaf primordium (Fig. 1). This zone, the shell zone, occurs along the basal and lateral limits of the bud. Vacuolation of adjacent cells and the shell zone delimit from the apical meristem a small group of meristematic cells which becomes the axillary bud apex.

During the next plastochron the bud primordium becomes dome shaped. Only anticlinal divisions occur in the outer layer, the tunica; periclinal divisions occur in the second layer of cells. Growth, in volume, of the bud primordium is by divisions in various planes of the corpus cells. Zonation of the corpus, as described for the vegetative shoot apex, becomes obvious late in the same plastochron or early in the next plastochron. By the time the zonation of the corpus has become established, the diameter of the bud primordium approaches the average diameter of 80μ of the vegetative shoot apex.

The first bud scale primordium is initiated by the axillary bud apex associated with the fifth youngest leaf primordium, or occasionally with the fourth youngest leaf. In all observed axillary buds, the first bud scale was formed on the adaxial flank of the axillary bud apex. A shell zone is often seen low on the abaxial flank. In *Cercis canadensis* numerous superposed axillary buds are produced. In one case seven superposed buds were observed in the axil of a single leaf. Observations of axillary buds are limited to the first formed bud which is the largest and is positioned above and adaxial to the second and subsequent buds in the axil of the same leaf. Figure 7 shows

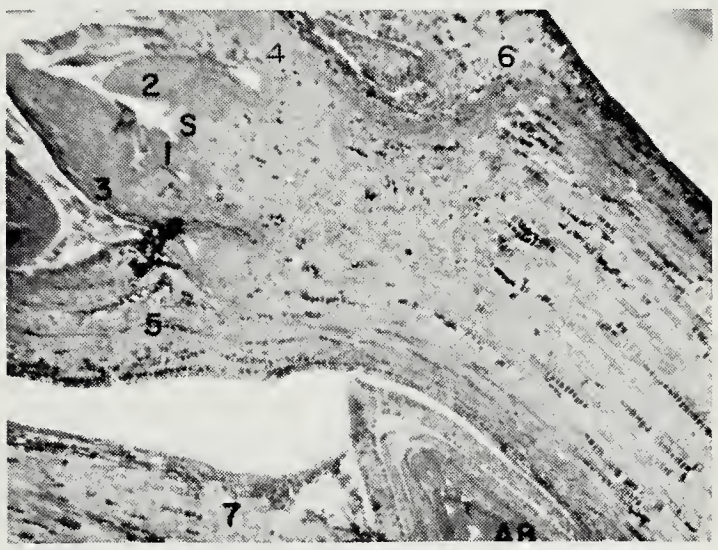

FIGURE 7. — Longisection of a vegetative shoot apex as well as the seven youngest leaf primordia showing various stages of bud development. The leaf primordia are numbered. S—shoot apex; AB—axillary bud. X70.

a vegetative shoot apex as well as the seven youngest leaf primordia in the axils of which are buds in various stages of development.

The relatively younger vegetative shoot apex of the axillary bud seems to be much more active in the initiation of bud scale primordia than the relatively older apical vegetative shoot apex is in initiating leaf primordia. Note in Figure 7 that the axillary bud associated with the seventh youngest leaf primordium has

at least eight bud scales. These eight bud scales were apparently initiated while the vegetative shoot apex was initiating three leaf primordia since the axillary bud in the fourth youngest leaf primordium has no bud scales. In the axil of the seventh youngest leaf primordium, the bud appears to be completely differentiated since several spirally arranged bud scales are present and the procambium is continuous with the procambium of the main shoot axis. No indication of the development of an axillary bud in the axil of any of the leaf primordia within an axillary bud was observed.

FIGURE 8. — Longisection of an axillary leaf bud associated with the seventh youngest leaf primordium. Note also the presence of a superposed bud. A—axillary bud apex; S—axillary bud scale; SB—superposed bud; PC—procambium. X70.

The Promeristem of Flower Buds. The leaves and flowers of *Cercis canadensis* are borne in separate buds. In early summer, a macroscopic examination of all buds does not allow precise determination of which are floral, but by mid to late summer the flower buds have developed sufficiently to be macroscopically distinguishable.

Identification of flower buds from serial longitudinal sections is not difficult. Flower buds contain numerous spirally arranged bud scales with a promeristem in the axil of each. Each promeristem will develop into a single, complete, perfect flower which will open the following spring. Comparison of Figure 9, a flower bud, with Figure 8, a leaf bud, shows three distinctive differences in leaf and flower buds. First, the flower bud is considerably larger. Second, bud scales are more numerous in flower buds. Third, only one promeristem occurs in a leaf bud but promeristems are numerous in flower buds. All the flower bud scale primordia originate from the apical meristem of the flower bud in the same manner that a leaf bud scale primordium originates from the leaf bud apex. A floral promeristem originates in the axil of the second youngest flower bud scale primordium in the same way an axillary bud primordium is initiated in the axil of a leaf primordium.

FIGURE 9. — Longisection of a flower bud collected in early July showing three floral promeristems and numerous flower bud scales. X70.

Until mid-July, the flower bud promeristem, as illustrated in Figure 10, cannot be microscopically distinguished from the vegetative shoot apex or the axillary bud apex on the basis of shape, size, tunica, or zonation of the corpus. The only distinguishing characteristic of the flower bud promeristem is the lateral organs produced. The first two primordia differentiate into bracts rather than leaves or bud scales. These bracts overlap and cover the promeristem, though not tightly.

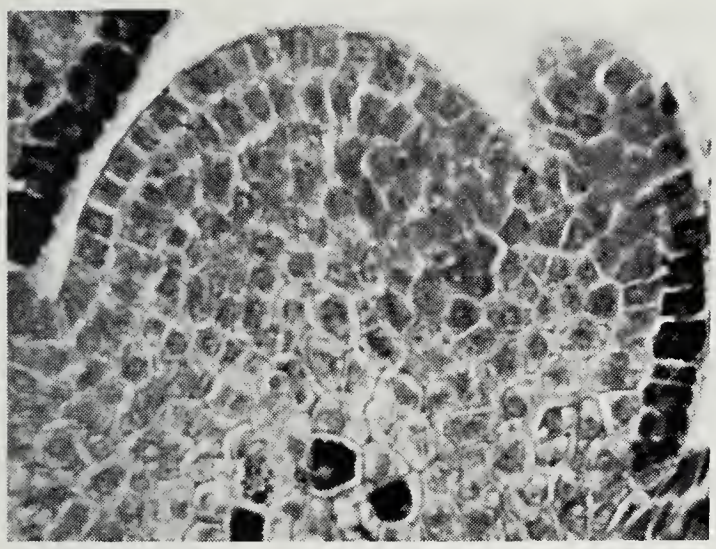

FIGURE 10. — Longisection of the apical promeristem of a flower bud collected in July. Note the zonation of the apex and the formation of a flower promeristem in the axil of the third youngest bud scale. X800.

The production of the next two lateral organs in mid-July is accompanied by a change in the shape, size, and organization of the flower bud promeristem (Fig. 11). The apex becomes wider and nearly flat rather than dome shaped. The tunica layer remains discrete and, as described in all other apices of *Cercis canadensis,* the second and third mantle layers exhibit some periclinal divisions. The peripheral zones are no longer curved but are composed of four to six columns of dark

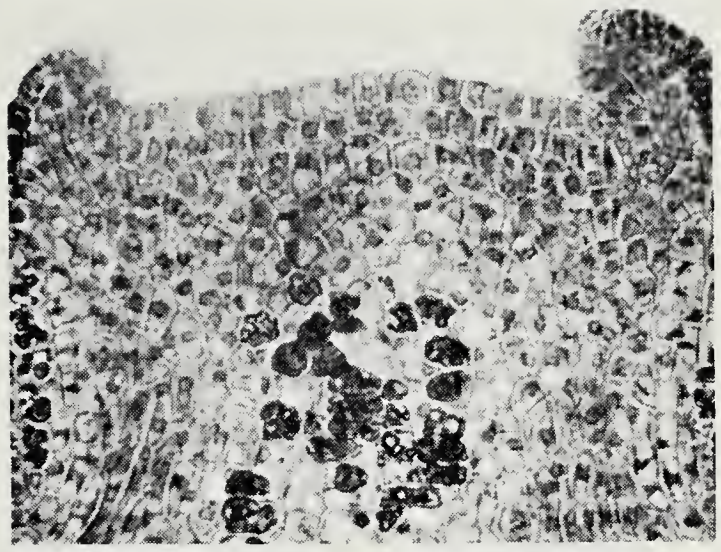

FIGURE 11. — Longisection of a floral promeristem after the initiation of the first two floral parts. Note the increased size, the reduction in the height of the dome, and the change in the zonation of the apex. Periclinal divisions are visible in the second layer. X800.

staining cells along both sides of the rib meristem. The rib meristem cells have elongated and the number of files of cells have increased to eight to ten. The initiation of floral organs beyond the first two primordia, presumably sepals, and further changes in the flower bud promeristem were not investigated. It is interesting to note, however, that all flower buds collected during the year show some promeristems which have ceased development soon after the development of the bracts. Even in the mature flower cluster can be found the imbricate bracts which enclose no visible floral parts.

Discussion

The vegetative shoot apex of *Cercis canadensis* resembles that of other investigated members of the Leguminosae in having a dome shape. The apex is smaller than any of the other investigated genera of the family. Its average diameter of 80μ is the lower limit of the range reported for *Glycine max* (Sun, 1957, p. 163),

the smallest diameter previously reported in the family. Boke (1940, p. 77) reported that the height of the apex of *Acacia longifolia,* the shortest previously reported apex, varies from 21 to 77μ. The average height of 21μ, with a maximum of 44μ, of *Cercis canadensis* ranks its as the shortest apex in the family.

When determining sizes of apices, measurements are traditionally measured above the last formed leaf primordium. By using this method of measurement, apices show an increasing size during each plastochron. However, a somewhat distorted view of the apex size can result because the measured height of the promeristem may include only a small proportion of the promeristem region, often no more than the outer layer of cells. This is because the portion of the shoot apex which extends above the youngest visible leaf primordium is determined by the location of the leaf primordium on the promeristem. When leaves are initiated low on the flanks as in *Agropyron repens* (Sharman, 1945) a considerable proportion of the promeristem is above the young leaf primordium. However, problems of measurement arise when the leaf primordium is initiated high on the flanks of the shoot apex as in *Cercis canadensis* or when the apex shape is low domed or flat as in *Hibiscus syriacus* (Tolbert, 1961, p. 250). Problems of measuring shoot apices were encountered by Rouffa and Gunckel (1951a, p. 295) and problems concerning the difficulty of determining shoot apex sizes were discussed by Gifford (1954, p. 499). Esau (1965, p. 105) mentions the effect of the level of leaf primordium insertion on the shoot apex in relation to changes in shape of the apex during a plastochron. A comparison of Figures 1 and 4 will show that the promeristem regions in both apices are very similar but measurements above the youngest visible leaf primordium results in measuring only two cell layers in Figure 1 and in Figure 4 includes only the mantle and central mother cell zone.

The data obtained from the currently used method of determining what portion of the apex to include in measurements tend to indicate that the meristematic tissue is "used up" during the formation of a primordium and that during the next plastochron an almost entirely new apex is formed. Study of the apices used in this investigation indicates that the leaf primordium results from the production of additional cells and that the apex size after leaf initiation is not as changed as the measurements indicate. These statements are not intended to imply that the apex is a static entity for it is fully realized that it is a dynamic system as has recently been illustrated by Soma and Ball (1964). A method of determining the size of the entire shoot apex, meristematic initials and their immediate derivatives, regardless of the level of the insertion of the leaf primordium is much needed.

The Tunica-Corpus Theory is applicable to the shoot apex of *Cercis canadensis.* In this paper the term "tunica" is used in the strictest interpretation, "discrete, independent, and self-perpetuating surface layer whose cells divide exclusively in anticlinal planes" (Popham, 1951, p. 260). *C. canadensis* has a single

tunica layer. A single tunica layer was also reported by Slade (1957, p. 283) for *C. siliquastrum* and by Hostetter (1965, p. 276) for the mature dormant plumule of *C. canadensis*. No other investigated genus of the Leguminosae typically has one tunica layer; however, it is apparent that not all workers use the term in its strictest interpretation. It would be interesting to determine if one tunica layer is characteristic in all members of the genus *Cercis*. A tunica composed of a single layer is not common among the angiosperms, especially the dicotyledons. In a table prepared by Gifford (1954, p. 518), of the 172 species listed only 39 have a one layer tunica; 23 of these were reported for the 49 species of monocotyledons included in the table.

The present study of the shoot apex of *Cercis canadensis* shows that it is an addition to the growing list of angiosperms whose apex exhibits a pattern of zonation superimposed upon a tunica-corpus organization (Philipson, 1947, 1954; Popham and Chan, 1950; Popham 1951, 1960; Gifford, 1954; Sun, 1957; Clowes, 1961; Tolbert, 1961; Romberger, 1963). In the family Leguminosae reports of zonation have not been frequent. Only the apex of *Glycine max* (Sun, 1957, p. 164) has been described as being definitely zoned. In *Acacia longifolia,* Boke (1940, pp. 77, 79) reported that "The apex of the corpus is dominated by a group of cells which may be termed initials" and "the inner corpus cells give rise to the rib meristem". Stratification of the flanks in the shoot apex of *Pisum sativum* was reported by Reeve (1948a, p. 71) but in the same species, Mitra (1950, p. 212) observed no "evident zonation".

Although many names have been applied to the zones of the shoot apex, the terms of Popham and Chan (1950, p. 480) are used in this paper. The shoot apex of *Cercis canadensis* exhibits Type VII zonation, the Usual Angiosperm Type of organization, described by Popham (1951, p. 260). This is the same type of zonation described by Sun (1957, p. 164) for *Glycine max.*

Soma and Ball (1964) have shown that centrally located cells on the surface of the shoot apex of *Lupinus albus* are shifted toward the flanks during growth. This experiment illustrates that zonal patterns should be considered as descriptive devices and should not lead to a belief in a permanent cell population within the shoot apex.

Leaf initiation in *Cercis canadensis* is first indicated by periclinal divisions in the second layer of cells. In terms of the Tunica-Corpus Theory this is the outer layer of the corpus and in terms of Type VII zonation this is layer two of the mantle. There seems to be no general pattern of leaf initiation with respect to the tunica-corpus organization in the shoot apex of investigated members of the family Leguminosae. Leaves may be initiated in the "subepidermal layer" as described by Slade (1957, p. 283) for *Cercis siliquastrum* which has a single tunica layer. Three different locations of leaf initiation are cited for the Leguminosae apices with two tunica layers: (1) in *Lupinus albus,* the second tunica layer (Ball, 1949, p. 441); (2) in *Glycine max,* the outer corpus layer (Sun, 1957, p. 166); and (3)

in *Pisum sativum,* the second tunica layer and outer corpus layer (Mitra, 1950, p. 213). In *Acacia longifolia,* with three tunica layers, both the tunica and corpus are involved in leaf initiation (Boke, 1940, p. 79). Apparently the only uniform feature of leaf initiation, not only among the members of the Leguminosae but also of other dicotyledons, is that periclinal divisions usually do not occur in the outer layer of cells (Clowes, 1961, p. 35).

Procambium development of *Cercis canadensis* resembles that of most of the other investigated members of the family Leguminosae as well as most dicotyledons in that the direction of differentiation is acropetal. Hostetter (1965, p. 271) reported that in the mature dormant plumule of *Cercis canadensis* procambium appears to differentiate acropetally. Ball (1952, p. 179) reports that procambium differentiation in *Lupinus albus* is basipetal after surgical isolation of the stem tip from subjacent tissue.

The vegetative shoot apex of the seedling was found to vary from the vegetative shoot apex of the mature tree only in size. The seedling apex was slightly larger than that of the mature tree. Reeve (1948a, p. 74) found that the shoot apices of fully developed embryos, seedlings and adult plants of *Pisum sativum* differ little from one another. The adult shoot apex is more highly arched and has an increased number of tunica layers as compared to the fully developed embryo (Reeve, 1948b, p. 600). Apparently few investigations of this type have been carried out using dicotyledons. Among the monocotyledons, Abbe, et al. (1951, p. 751) has shown that the shoot apex of *Zea mays* increases exponentially in all dimensions affecting area. Apex zonation and cell size did not change. Ball (1941, p. 831) demonstrated a change from Type VII zonation, the Usual Angiosperm Type, of the seedling to Type VI zonation, the Opuntia Type which includes a cambium-like zone of cells, of the adult plants of *Phoenix canariensis.* The volume of the apex of *P. canariensis* decreased during development of seedling to adult plant. Similar changes in zonation and apex size has been reported for gymnosperms such as *Pseudotsuga taxifolia* (Allen, 1947, p. 208). It should be noted, however, that the study of the shoot apex of *Cercis canadensis* seedlings is only a preliminary study.

No differences in shape, size, or organization between the vegetative shoot apex and the axillary bud apex was found in *Cercis canadensis.* Sun (1957, p. 171) reported that in *Glycine max* the axillary bud apex was of smaller diameter than the vegetative shoot apex; no differences in apex organization were evident. The formation of the axillary bud apex in both *Glycine max* and *Cercis canadensis* followed the pattern described by Garrison (1949, p. 213) for *Syringa vulgaris* in which the bud apex is derived directly from the apical meristem of the main shoot being delimited by the formation of shell zone.

Rouffa and Gunckel (1951a, p. 297), in a study of the shoot apices of 54 species of the family Rosaceae, found a wide range of variation in apex sizes and concluded that "Much of the difference between active shoots and perennating buds may be

due to the perennating buds usually being axillary, but the range of variation is probably still significant.'' In five species, *Neillia sinensis* Oliv., *Stephanandra Tanakae* French., *Cotoneaster horizontalis perpusilla* Schneid., *Adenostoma sparsifolium* Torr., and *Prunus Laurocerasus Schipkaensis* Spaeth., Rouffa and Gunckel (1951b, p. 303) found that both the active shoots and perennating buds showed pith development typical of long shoots with cells arranged in long files. This type of pith development is also found in *Cercis canadensis.* A seasonal study of the vegetative shoot apex in *Hibiscus syriacus* conducted by Tolbert (1961, p. 254) correlated seasonal changes, in terms of long and short shoot development and dormant terminal buds, with pith rib meristem changes. The dormant shoot apex of *H. syriacus* is described as looking ''as though it were arrested during the process of active growth'', a description which also applies to *Cercis canadensis.*

Borthwick and Parker (1938) in a study of the influence of photoperiods upon the differentiation of meristems and the blossoming of Biloxi soybeans recorded some information concerning flower development. This is the only report on flower development of a member of the family Leguminosae, although Sunderland (1961) did make measurements of the rate of cell multiplication and expansion in the reproductive apices of *Lupinus albus,* but no information concerning the size, shape, or organization of the floral apex or the initiation of floral parts was given.

Borthwick and Parker (1938) did not describe the size or organization of the floral promeristem of soybean, but their investigation showed that the vegetative apex of soybean ceases producing leaves and rapidly initiates a series of spirally arranged small bracteal leaves. A bud develops in the axil of each bracteal leaf. After forming the primordia of a pair of prophylls, the promeristem of each bud gives rise directly to the various whorls of flower parts. Each bud gives rise directly to a single flower. This is essentially the same developmental sequence observed in each flower bud promeristem of *Cercis canadensis* except that the small bracteal leaves of soybean were developmental equivalents of flower bud scales in *C. canadensis.* The prophylls of soybeans are carried outward by the elongation of the pedicel whereas in *C. canadensis* the bracts remain at the base of the pedicel.

Summary

1. The vegetative shoot of *Cercis canadensis* terminates in a low dome shaped apex with an average diameter of 80μ and an average height of 21μ. The apex exhibits Popham's (1951, p. 260) Type VII zonation, the Usual Angiosperm Type of organization, consisting of a mantle, central mother cell zone, rib meristem, and peripheral zone. The tunica consists of a single layer of cells.

2. The leaf primordium is first indicated by periclinal divisions in the second cell layer.

3. Differentiation of the procambium is acropetal into the leaf from the stem procambium.

4. The seedling vegetative shoot apex differs from that of the mature tree only by being slightly larger.

5. Axillary buds, first indicated by a shell zone, are initiated adaxial to the second or third youngest leaf primordium. The shape, size, and organization does not differ from the vegetative shoot apex.

6. The floral promeristem before initiation of floral parts does not differ in shape, size, or organization from the vegetative shoot apex. The initiation of the first floral parts produces marked changes in the shape, size, and organization of the floral apex.

Acknowledgements

The author wishes to express appreciation to the following individuals for their assistance in making this study possible: Dr. Robert D. Henry, Western Illinois University, for his assistance, suggestions, and guidance; Western Illinois University Audio-Visual Department and Sherwin L. Kroll, Springfield High School, for aid with photography; and Dr. Donald Galitz, Western Illinois University, for technical aid.

This study represents a portion of a thesis submitted in partial fullfillment of the requirements for the Master of Science degree at Western Illinois University. Partial support during this study was received through a National Science Foundation Fellowship in the program of Summer Fellowships for Secondary School Teachers, N. S. F. Fellowship Number 72177.

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Manuscript received Sept. 18, 1967.

PILOCARPINE MODULATION OF AN EFFECT OF CHLORPROMAZINE ON MOUSE BEHAVIOR

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ABSTRACT. — Pilocarpine, given subcutaneously after prior administration of atropine methyl bromide, enhances the induction of loss of righting reflex without true general anesthesia caused by chlorpromazine in mice. The same dose of pilocarpine failed to exert this effect when atropine sulfate was substituted analogously for the atropine methyl bromide.

Chlorpromazine has been shown to produce a depression in mice which can cause loss of the righting reflex (Burton, Sodd and Goldin, 1957). Utilizing three different test systems, Proctor and his co-workers (1964) have demonstrated extension of the action of the phenothiazine tranquilizer, perphenazine, caused by low dosages of organophosphorous anticholinesterase agents in mice. The action of the anticholinesterase agents in producing this enhancement of tranquilizer action was accomplished under conditions which best support a concept that their effect was exerted by a cholinergic action on the central nervous system (CNS). These reports have engendered interest on our part in the question as to whether or not a receptor - active, CNS - active cholinergic agent such as pilocarpine (Pfeiffer and Jenny, 1957) could enhance the ability of chlorpromazine to abolish the righting reflex of mice, and, if so, whether or not such enhancement could be associated with a probable CNS site of action. The present communication presents the results obtained when the pilocarpine effect on chlorpromazine induction of the loss of the righting reflex in mice was observed under the influence of the anticholinergic agents, atropine methyl bromide and atropine sulfate. Atropine methyl bromide was given under conditions so as to expect it to exert only peripheral anticholinergic action, while atropine sulfate was given in a way whereby it could be expected to yield both CNS and peripheral action (Pfeiffer and Jenny, 1957; Proctor et al. 1964; Goodman and Gilman, 1965; Stein, 1963). These atropine derivatives were employed with a view toward influencing any pilocarpine effect ascertained by the respective distribution patterns of their anticholinergic action.

MATERIALS AND METHODS

The studies were carried out in adult albino mice weighing 20-30 g. Drugs were utilized in the following forms: chlorpromazine hydrochloride, pilocarpine nitrate, atropine sulfate, and atropine methyl bromide. All dosages have been expressed in terms of the drug salt used. Distilled water solutions of the drugs used for injection were made up fresh on the day of use. Pilocarpine was administered subcutaneously. All the other drugs were given intraperitoneally. Subcutaneous injections were made in a volume of 0.005

ml/g; intraperitoneal injections in a volume of 0.01 ml/g. Each animal, treated or control, received the same number and volume of injections on a similar time schedule and by analogous routes, using isotonic saline as a "sham" injection where necessary. Time schedule for injection of drugs, indicated in minutes prior to making the first test for loss of righting reflex, was as follows: chlorpromazine, 30 min; atropine methyl bromide or atropine sulfate, 10 min; pilocarpine, 0 min (i. e., immediately before the first test of the reflex). The animals were tested for loss of the righting reflex at ten minute intervals over a period of two hours. Loss of the reflex was assumed when present for one or more minutes. Any animal failing to spontaneously exhibit return of the reflex was left undisturbed and was included in the total of animals having loss of righting reflex for the given interval of time being assessed. Each experimental group consisted of 10 mice. Statistical significance of the data was assessed for each 10 min time interval by X^2_c determination. Significance of differences was set at or below the 5 per cent probability level.

Results

In running preliminary experiments designed to locate dosages of chlorpromazine which would be useful for testing the pilocarpine enhancing effect an interesting phenomenon was observed. Beginning with the 25 mg/kg chlorpromazine dose (used originally by Burton, Sodd and Goldin, 1957) and testing several lower dosages of chlorpromazine, it was found that the tranquilizer could cause loss of the righting reflex without inducing true anesthesia. Animals responding in this way tried to escape when righted and prodded, and attempted to bite objects brought near their heads. Such behavior differs from that of animals which are in the surgical state of anesthesia and presents the possibility that the mice were merely "indifferent" to being placed on their backs. This kind of behavior, in contrast to a state more nearly approximating true surgical anesthesia, was most marked at 10 mg/kg dose of chlorpromazine, dosages of 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg and 25 mg/kg of the tranquilizer having been tried. At the 10 mg/kg dose every animal in the group of 10 animals tested elicited the kind of behavior in question at some time during the two hour test period and none of them was ever truly anesthetized. Higher dosage effects were less readily distinguishable from general anesthesia. The 5 mg/kg chlorpromazine dosage failed to produce loss of righting reflex in any animal over the same period of observation in the trial run.

Figure 1 presents the results obtained when the 5 mg/kg chlorpromazine dosage in mice was subjected to the influence of 11 mg/kg pilocarpine administered subsequently to either atropine methyl bromide or atropine sulfate. It allows comparison of these findings with those obtained from groups of animals receiving 10 mg/kg chlorpromazine; 5 mg/kg chlorpromazine; 5 mg/kg chlorpromazine plus 20 mg/kg atropine MeBr; and saline-injected controls, respectively.

It is apparent that 11 mg/kg pilocarpine, given subsequently to 20 mg/kg atropine methyl bromide and 5 mg/kg chlorpromazine (curve II), produced considerable enhancement of the capacity of 5 mg/kg chlorpromazine to induce loss of the righting reflex (curve III). Loss of the reflex did not represent the surgical stage of anesthesia in these animals. This enhancement converted a vir-

tually subthreshold 5 mg/kg dosage of the chlorpromazine to about the same level of effect elicited from 10 mg/kg chlorpromazine given alone (curve I). It can also be seen that when 5 mg/kg atropine sulfate replaced the 20 mg/kg atropine methyl bromide as the anticholinergic agent given prior to the 11 mg/kg pilicarpine, the latter was no longer able to enhance the 5 mg/kg chlorpromazine effect on the reflex (curve IV (a)). A similar inability was exhibited (a) by 20 mg/kg atropine methyl bromide given with the chlorpromazine but without the pilocarpine (curve IV (b)), and (b) by the saline controls (curve IV (c)).

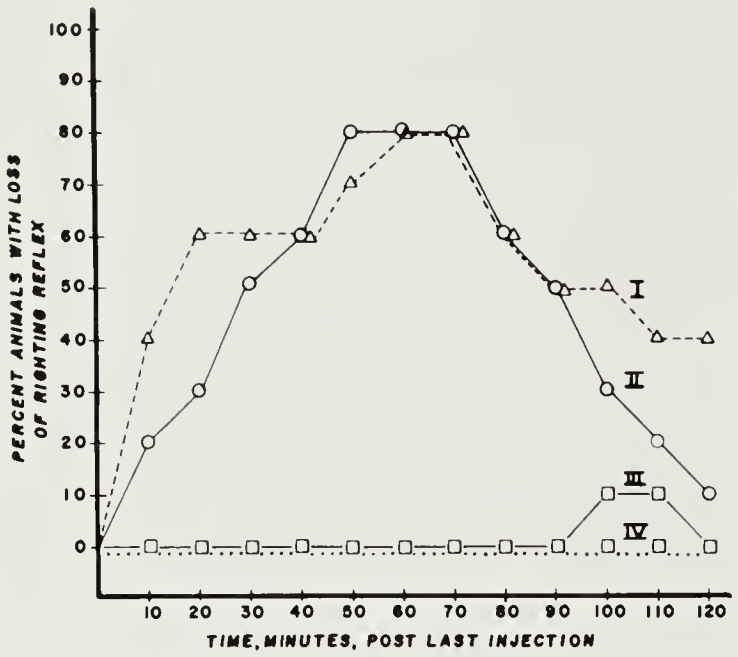

FIGURE 1. — Time course of the effect of 11 mg/kg pilocarpine (pilocarp.) on loss of righting reflex in groups of 10 mice pretreated with 5 mg/kg chlorpromazine (CPZ) and either 20 mg/kg atropine methyl bromide (AtMeBr) or 5 mg/kg atropine sulfate ($AtSO_4$). Experimental curves obtained from animal groups (n=10) receiving other dosage regimens are presented for comparison.

Key:

Curve I:
10 mg/kg CPZ

Curve II:
5 mg/kg CPZ + 20 mg/kg AtMeBr + 11 mg/kg pilocarp.

Curve III:
5 mg/kg CPZ

Curve IV:
Curve obtained for each of the following:
(a) 5 mg/kg CPZ + 5 mg/kg $AtSO_4$ + 11 mg/kg pilocarp.
(b) 5 mg/kg CPZ + 20 mg/kg AtMeBr
(c) Saline controls

p values, X^2_c: In comparing per centage values from either Curve I or II with either Curve III or IV at a given time interval: 50 and 60% response, $p < 0.05$; 70 and 80% response, $p < 0.01$; 40% response and lower, $p > 0.05$. In comparing Curve I with Curve II, $p > 0.1$ at all time intervals.

Discussion

The experiments performed appear to demonstrate that under certain conditions pilocarpine can enhance the effect of chlorpromazine in causing loss of the righting reflex without inducing true anesthesia in mice. The pilocarpine effect is apparently exerted when it is given subsequent to prior administration of atropine methyl bromide but not when it is given following prior administration of atropine sulfate. The pattern of influence of these two anticholinergic agents on this pilocarpine modulation of chlorpromazine effect would make it appear that the response to pilocarpine is more related to a CNS action than to a peripheral action of pilocarpine.

Reversal of the modulation was effected by atropine sulfate but not by atropine methyl bromide when each was given under conditions so fixed as to expect CNS and peripheral anticholinergic action from the former but only peripheral action from the latter (Goodman and Gilman, 1965; Pfeiffer and Jenny, 1957; Proctor et al., 1964; Stein, 1963). The compara-

tive effect of atropine sulfate and atropine methyl bromide on the pilocarpine extension of chlorpromazine effect which has been observed exhibits a degree of similarity to the effect of pilocarpine on the pole climbing conditioned response in the rat first observed by Pfeiffer and Jenny (1957). These investigators found that pilocarpine inhibited this conditioned response when given after atropine methyl bromide but not when given after atropine sulfate.

The present findings are also compatible with a working hypothesis which has been advanced earlier (Proctor, et al., 1964). This hypothesis states that tranquilizer activity may be due in part to a depression of CNS adrenergic activity, caused either by blockade (Marrazi, 1953, 1957) or by reduction in CNS neurohumor level (Brodie, et al., 1960; Costa, 1960), with a resultant relative ascendency of cholinergic activity which contributes to tranquilizing action.

The present data provides evidence that pilocarpine, a CNS-active cholinergic agent (Pfeiffer and Jenny, 1957; Pfeiffer, 1959), can enhance a gross tranquilizer response due to chlorpromazine, a tranquilizer known to block the action of adrenergic amines on CNS synapses (Marrazi, 1957), under conditions which best suport interpretation of a CNS site of action for the pilocarpine. It is believed that such data yield support for the working hypothesis which has been advanced previously (Proctor, et al., 1964), even though it is understood that the data provide whole animal unitary evidence in support of this speculation rather than analytical evidence for the specific parts of the CNS that may be involved in the behavioral effect observed.

Acknowledgments

This work was supported in part by a grant from the Ernest T. Collins fund by research grants 5-SO1-5422-05 and 1-SO-1-FR-05341 from the National Institutes of Health, U.S. Public Health Service.

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Manuscript received Oct. 27, 1967.

FOSSIL MUSK OXEN OF ILLINOIS

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ABSTRACT. — The first definitely identifiable specimens of *Ovibos* from Illinois are reported from Henderson and Fulton Counties. Eleven other locality records, six previously unpublished, for musk oxen in Illinois, are reviewed, representing *Symbos*, ovibovines of uncertain generic identity, or possibly non-ovibovine bovids.

The purpose of the present communication is to place on record the first definitely identifiable specimens of the living musk ox genus *Ovibos* known to us from Illinois, and to review present knowledge of fossil ovibovines in the Pleistocene of Illinois. Following is an annotated list of all specimens known to us. The numbers (1-13) preceding the account of each specimen or specimens from a given locality correspond to the numbers by which localities are designated on the distribution map (Figure 1). All specimens have been examined by one of us (Ray), excepting those from localities 9 and 13.

ANNOTATED LIST

1. *Ovibos* (?)*moschatus. Oquawka, 5.5 miles east southeast of, Henderson County.* The locality is in the bed of the south branch of Smith Creek (Jinks Hollow on U.S. Geological Survey topographic map, 15′ ser., Oquawka quadrangle), SW¼, Sec. 33, T. 11 N., R. 4 W. The location can not be ascertained more precisely owing to the death of the collector, Mr. Wilford Peterson.

This record is based upon a partial skull collected by Mr. Peterson in 1965 and turned over to the Department of Geology, Monmouth College. The specimen was forwarded to the U. S. National Museum for further study and permanent deposit, where it has received the number U.S.N.M. 23456 in the Division of Vertebrate Paleontology. Casts have been placed in the collections of Monmouth College, the Field Museum of Natural History (PM 14680), and the National Museum of Canada.

The specimen consists of the braincase and the interorbital region of the frontals. The free, projecting portion of each horn core has been lost, and the entire specimen has been abraded, apparently by stream-tumbling (Figures 2 and 3). The specimen compares well with crania of adult bulls of modern *Ovibos moschatus*, except in its unusually great postorbital breadth of 171.5 mm. (least width posterior to orbits and anterior to horn cores), as compared to postcornual breadth of 140.5 mm. (least width immediately posterior to horn cores). These may be contrasted to similar measurements of 139.6 mm. and 132.0 mm. in a modern adult bull of *O. moschatus* (U.S.N.M. 288025, Division of Mammals). The specimen from Fulton County (locality 3) is too abraded and broken to permit accurate measurement. The maximum postorbital breadth among 85 skulls of Recent adult male *Ovibos moschatus* measured by Tener (1965, Table 44) is 156.0 mm. The fossil from Henderson County may be referred with confidence to *Ovibos*, but only with question to *O. moschatus*.

Illinoian till, Peorian loess and Recent alluvium are the only surface materials in the Jinks Hollow drainage basin, i.e., the collecting locality. Classification of the glacial materials is based on lithologic correlation with Horberg's section 9a (1956, p. 32), located 14 miles north-

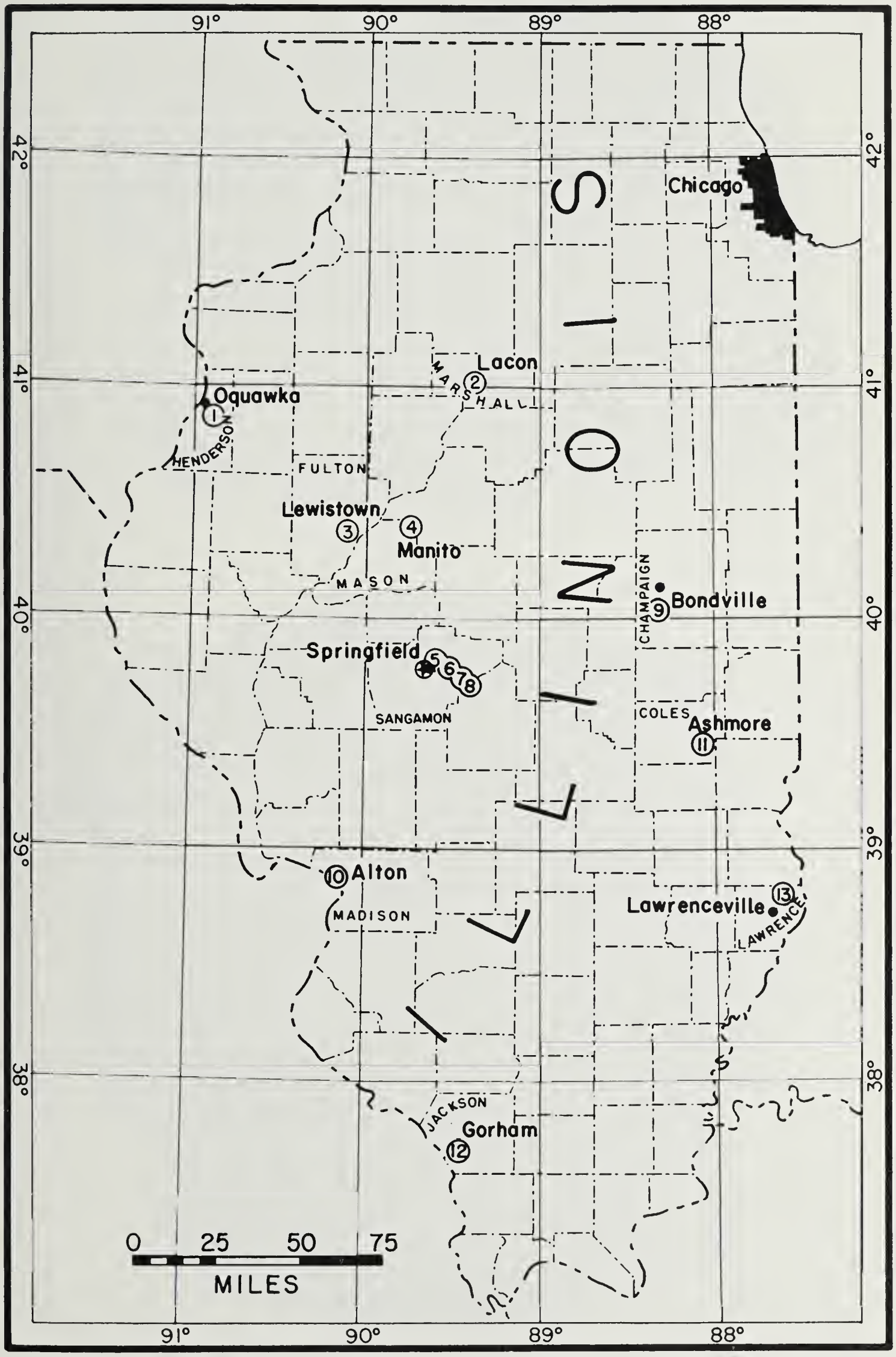

FIGURE 1.—Distribution of fossil musk oxen in Illinois. Encircled numbers (1-13) designate localities and correspond to numbered paragraphs in the ANNOTATED LIST.

FIGURE 2.—Cranium of *Ovibos* (?) moschatus, U.S.N.M. 23456, from Henderson County, Illinois, in posterior (A) and dorsal (B) aspects. Approximately X 3/8.

FIGURE 3.—Cranium of *Ovibos* (?) *moschatus*. U.S.N.M. 23456, from Henderson County, Illinois, in left lateral (A) and ventral (B) aspects. Approximately X 3/8.

east of Jinks Hollow. The till is yellowish-brown, clayey, sandy, silty, and locally gravelly or pebbly. Up to 40 feet of till is exposed in the valley wall. The upper surface of the till is locally oxidized, suggesting that late Illinoian or early Wisconsian erosion partially removed the weathering profile. Yellowbrown clayey loess overlies the till.

The permineralized character of the braincase would seem to preclude the possibility that the musk ox was buried initially in the Recent alluvium. Material resembling till was scraped from depressions in the skull. The clay matrix was separated from the coarser fraction and x-ray diffraction analysis of the clay was made in an attempt to correlate the relative amounts of illite, montmorillonite, kaolinite and chlorite with those in local tills. Although no conclusive results were obtained, the nature of the material adhering to the skull suggests that it was derived from Illinoian till. Thus, it seems possible that the *Ovibos* specimen could be of Illinoian age.

2. *Ovibovini, gen. et. sp. indet. Lacon, Marshall County.* No further data are available regarding locality.

This record is based on the centrum of a posterior cervical vertebra (probably the seventh) of an undetermined ovibovine in the collections of the Field Museum of Natural History (PM 15227). The specimen was collected by Mr. Magoon Barnes and was given to the museum in 1936.

3. *Ovibos moschatus. Lewistown, 3.8 miles southeast of, Fulton County.* This locality is immediately west of the Morton School on a ridge known locally as "Hill Top," bordered on the east by the west bluff of the Illinois River and on the west by the valley of Coal Creek. It is near the midpoint, and perhaps 100 feet south of the northern boundary of the NW¼, NE¼, Sec. 6, T. 4 N., R. 4 E. (U.S.G.S. topographic map, 15′ ser., Havana quadrangle). The site has been located precisely by Cole and Deuel (1937, Figure 16 and Plate VIA.).

This specimen consists of a much abraded and fragmented braincase with the bases of the horn cores of a presumably young adult male with occipital sutures open (Figures 4 and 5). It was reported as *Symbos cavifrons* by Cole and Deuel (1937, pp. 73, 268), based upon field identification by Professor Alfred Romer, but its characters clearly are those of *Ovibos*, rather than of *Symbos*. The narrow, dorsal, mid-sagittal groove separating the left and right horn cores and the wide, basin-shaped dorsal depression of the horn cores lacking a pronounced anterior interorbital extension (Figure 4), immediately indicate *Ovibos*. There is nothing in the preserved portion of the skull to separate it from *O. moschatus*.

The specimen was until recently housed in the collections at the Dickson Mounds, Museum of the Illinois Indian, near Lewistown, Fulton County, but has now been presented by Mr. Marion Dickson to the Illinois State Museum where it has received the catalog number I.S.M. 416118.

It was found beneath mound F° 10 in the Morton Group of Cole and Deuel, 1.5 feet down in the undisturbed loess which mantles the "Hill Top" ridge. The loess here is undoubtedly the Peoria Loess of late Wisconsinan age (Frye, Willman, and Black, 1965, p. 56).

4. *Symbos cavifrons. Manito, Mason County.* According to Hay (1923, p. 253), "the exact location of the skull was in section 22, township 23 north, range 6 east [actually west] . . . ." (U.S.G.S. topographic map, 15′ ser., Manito quadrangle).

The specimen (U.S.N.M. 7800) is a well preserved braincase, with the horn cores and much of the interorbital and circumorbital regions, of *Symbos cavifrons* collected from a peat deposit of presumed Wisconsinan age. It has been reported upon previously by Hay (1923, pp. 253-254), who suggested that it might represent a female individual. On the basis of analogy with sexual dimorphism in *Ovibos moschatus*, it seems much more likely that it represents a young adult male.

5. *Ovibovini* (?), *gen. et sp. indet. Springfield, 6.5 miles east northeast of, Sangamon County.* The locality is the workings of the Clear Lake Sand and Gravel Company along the north shore of Clear Lake, SW¼, Sec. 22, T. 16 N., R. 4 W. (U.S.G.S. topographic map, 7½′ ser., Springfield East quadrangle), from which Holman (1966) has reported remains of fossil turtles.

In the collections of the Illinois State Museum (uncatalogued) is the well preserved crown of a left M_3 lacking most of the roots. The tooth is almost certainly ovibovine, but not further identifiable in the present state of knowledge. It is somewhat atypical of ovibovine molars in the presence of a well devel-

FIGURE 4.—Cranium of *Ovibos moschatus*, I.S.M. 416118, from Fulton County, Illinois, in posterior (A) and dorsal (B) aspects. Approximately X ⅜.

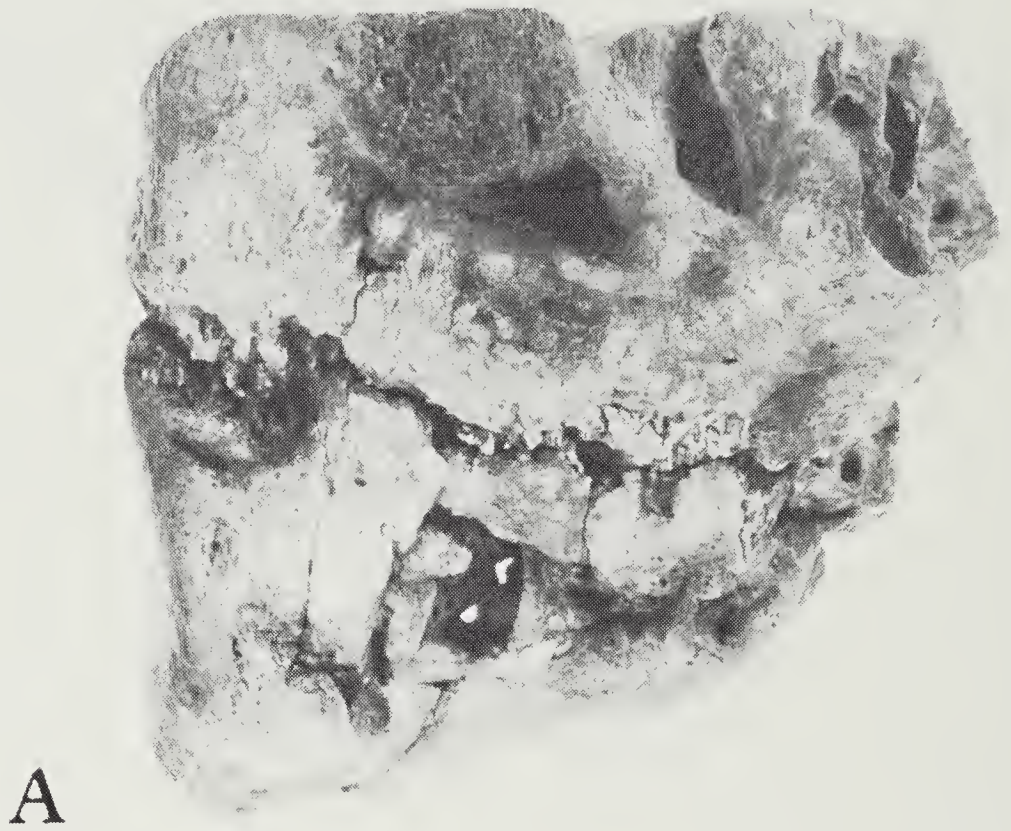

FIGURE 5.—Cranium of *Ovibos moschatus*, I.S.M. 416118, from Fulton County, Illinois, in right lateral (A) and ventral (B) aspects. Approximately X 3/8.

oped labial stylid. The occlusal length of the tooth is approximately 51 mm., the greatest length of the crown parallel to the occusal surface approximately 56 mm., and the greatest breadth of the crown 25.2 mm.

The collections of the Field Museum of Natural History contain a cast (P 27139, referred to *Euceratherium*) of a left M^2 from the Clear Lake Gravel Pit. The original specimen is in the Illinois State Museum according to the records of the Field Museum, but is not now to be found there. The cast represents a large tooth (maximum occlusal length 38.0 mm., maximum crown breadth 33.9 mm.) which compares closely to the left M^2 in referred specimens of *Symbos cavifrons*.

6. *Symbos*(?), *sp. indet. Springfield, 4-5 miles east of, Sangamon County.* The locality is described in the records of the Field Museum of Natural History as a late Pleistocene gravel pit along the Mechanicsburg Road. Dr. Paul W. Parmalee (pers. comm.) feels that this locality is probably the pit of the Springfield Sand and Gravel Company in the bed of the Sangamon River, Sec. 36, T. 16 N., R. 4 W. (U.S.G.S. topographic map, 15′ ser., Mechanicsburg quadrangle) eight and one half miles east of Springfield, from which he (1967) has recorded *Cervalces*.

The specimen is a water worn fragment of the occipito-parietal region of the skull, tentatively referred to *Symbos*. It was collected in 1954 by Dr. Carlton Condit, then Head Curator of Geology at the Illinois State Museum, and in 1956 was sent as a gift to the Field Museum of Natural History where it has been catalogued as no. PM 1418.

7. *Symbos cavifrons. Springfield, 8.5 miles east of, Sangamon County.* The locality is a pit of the Sangamon Valley Sand and Gravel Company, about one fourth mile south of the Springfield Sand and Gravel Company pit (locality 6). The locality lies in the southwest corner of Sec. 36, T. 16 N., R. 4 W. (U.S.G.S. topographic map, 7½′ ser., Springfield East quadrangle).

The specimen consists of the posterior end of the braincase of an adult individual with much of the skull roof sheared away, apparently by excavating machinery, but retaining the proximal portion of the right horn core. It was found on August 20, 1967, by Mr. Robert D. Broughton, of Springfield "in a large gravel pool at an approximate depth of 65 feet [below ground level] and surrounded by a yellowish clay." The specimen was submitted to Dr. Paul W. Parmalee who forwarded it to one of us (Ray) for study. It has been returned to the finder.

8. *Ovibovini* (?), *gen. et sp. indet. Buckhart, Sangamon County.* The locality is the pit of the Buckhart Sand and Gravel Company, NW¼, Sec. 16, T. 15 N., R. 3 W. (U.S.G.S. topographic map, 15′ ser., Mechanicsburg quadrangle).

In the collections of the Illinois State Museum and of the Field Museum of Natural History (P 27138, referred to *Euceratherium*) are casts of an isolated crown of a right upper molar, either the M^1 or the M^2, measuring 34.4 mm. in maximum occlusal length and 26.3 mm. in maximum crown breadth. The casts compare favorably to the corresponding teeth of various ovibovines, including those from the Frankstown Cave, Pennsylvania, referred to *Bootherium bombifrons*, those of uncertain identity from Alton (locality 10 below), the M^1 of referred specimens of *Symbos cavifrons*, and the M^2 of the type specimen of *Euceratherium*(?) *americanum*, U.S.-N.M. 7622, the affinities of which remain uncertain.

9. *Symbos cavifrons. Bondville, approximately 4 miles south southeast of, Champaign County.* Hay (1923, p. 253) indicates that the locality is on the farm of John Busey, in Sec. 31, T. 19 N., R. 8 E. (U.S.G.S. topographic map, 7½′ ser., Bondville quadrangle).

According to Hay (1923, p. 253) the specimen is "the rear portion of the skull with the horn-cores of . . . *Symbos cavifrons*." It is number P6857 in the collections of the University of Illinois, where it is at present on display (Bader, pers. comm.).

10. *Symbos*(?), *sp. indet. Alton, Madison County.* Exact location unknown, but from a quarry in the vicinity of Alton.

William McAdams, of Alton, assembled a collection of Pleistocene mammals occurring in concretions within the loess near Alton (McAdams, 1884). This collection ultimately reached the U. S. National Museum where it was studied and reported upon by Hay (1920, pp. 109-117). Hay later (1923, pp. 254, 338-340, etc.) repeated the faunal list, with notes on certain species, and discussed further the geology and probable age of the source deposits.

Among some 12 bovid cheek teeth

from Alton other than those of *Bison*, Hay (1920, p. 115) tentatively referred one, a left M^2?, U.S.N.M. 9011, to his *Symbos promptus*, described in the same paper (p. 125) on the basis of a single left M^3 (U.S.N.M. 9120) and several fragmentary vertebrae from Afton, Oklahoma. The impropriety of referring a deeply worn, damaged lower molar to a species known only from an upper molar, not to mention the futility of erecting a new species of *Symbos* on the basis of an isolated tooth, is obvious. The tooth from Alton may be referred with question to the genus *Symbos*.

The remaining non-*Bison* bovid teeth discussed by Hay (1920, pp. 113-115; 11 teeth by his count, but 12 according to his text and to existing specimens, U.S.N.M. 9007-9009) were referred to *Taurotragus americanus* described by Gidley from Cumberland Cave, Maryland. Gazin (Gazin, 1933; Gidley and Gazin, 1938, pp. 86-90) tentatively transferred this species to the genus *Euceratherium* and suggested (1933) that the material from Alton might also be referable to *Euceratherium*(?) *americanum*. In the opinion of one of us (Ray) the material from Alton originally referred by Hay to *Taurotragus americanus*, together with poorly preserved, associated, right M_1-M_3(U.S.N.M. 9136), should be referred with question to *Symbos*. The occurrence of *Euceratherium* in the eastern United States has not as yet been established, and will remain doubtful until cranial material, preferably including horn cores, is discovered.

11. *Ovibovini, gen. et sp. indet. Ashmore, one mile south of, Coles County.* Gravel pits along Polecat Creek in the northern half of Sec. 6, T. 12 N., R. 11 E. (U.S.G.S. topographic map, 15′ ser., Oakland quadrangle).

Among fossils from a post-glacial alluvial gravel in these pits, Galbreath (1938, p. 308) identified as Ovibovinae, gen. et sp. indet., an anterior dorsal vertebra deposited in the Field Museum of Natural History (P 15273).

There is also an apparently ovibovine left innominate bone in the collections of the Field Museum of Natural History (PM 531). Data with the specimen indicate that it was collected from a gravel pit three miles south of Ashmore, and was received in 1936 as a gift from E. C. Galbreath (field no. ECG 3115). According to Galbreath (pers. comm.) this specimen comes from the same pit as the vertebra, does not represent a separate locality, and was in fact collected during 1941.

12. *Ovibovini, gen. et sp. indet. Gorham, Jackson County.*

This record is based on a partial cervical vertebra of an undetermined ovibovine collected near Gorham by a farmer in 1956 or 1957. The specimen was returned to the finder, but casts were retained in the collections of the Field Museum of Natural History (PM 2377) and the Illinois State Museum (I.S.M. 416117).

13. *Symbos cavifrons. Lawrenceville, 4 miles northeast of, Lawrence County.* The locality is a gravel pit in the NW¼, Sec. 27, T. 4 N., R. 11 W. (U.S.G.S. topographic map, 7½′ ser., Birds quadrangle), on the western border of the Lawrenceville-Vincennes Municipal Airport (formerly George Field).

The specimen is an axis, no. P 200 in the vertebrate paleontological collection of the Department of Zoology at Southern lllinois University, reported by Galbreath (1962) and identified as *Symbos cavifrons*. The age of the glacially derived source beds was determined as "much later in time than the Shelbyville glacier," which is to say that they are latest Wisconsinan in age.

Discussion

The 13 recorded finds of fossil musk oxen in Illinois include two of *Ovibos* (localities 1 and 3); four, more or less certainly identified, of *Symbos cavifrons* (localities 4, 7, 9, and 13); two questionably referred to *Symbos* (localities 6 and 10); and five of Ovibovini or Ovibovini (?), gen. et sp. indet. (localities 2, 5, 8, 11, and 12).

Although Adams (1923, p. 143) states "two musk ox are recorded from the state . . . , Symbos cavifrons and the modern Ovibos moschatus . . . ," and Bader and Techter (1959, p. 3) list *Ovibos* sp. among the fossil mammals of Illinois, we have been unable to find the basis for these statements, and assume therefore that the present records are the first

for Illinois. Additional finds of *Ovibos* and *Symbos* are to be expected, as are finds of *Bootherium,* thus far not recorded for Illinois, but known from nearby states (Michigan, Indiana, Kentucky, Missouri, and Nebraska).

Satisfactory field data are available only for the specimens from localities 3, 11, and 13 and the specimens from the last two of these localities are not very informative morphologically or taxonomically. There is as yet no basis other than the cranium with horn cores by which *Symbos* and *Bootherium* may be distinguished with certainty. Although dental material has been referred customarily to *Symbos,* or occasionally to *Euceratherium,* and dissociated postcranial material to *Symbos,* there has been no basis for excluding *Bootherium.* If authors have implicitly accepted the repeatedly suggested, but undemonstrated, congeneric status of *Symbos* and *Bootherium,* then *Bootherium* is the name with clear priority.

Thus the partial skull of *Ovibos moschatus* from Fulton County is the only specimen of fossil musk ox from Illinois that is both closely identifiable and accompanied by adequate field data. Further advances in knowledge of fossil musk oxen in Illinois and in general will depend on the discovery of well preserved material, circumspect identification, and the recording of adequate geographic and stratigraphic data.

Acknowledgments

We wish to express our thanks to Drs. R. S. Bader (University of Illinois), E. C. Galbreath (University of Southern Illinois), P. W. Parmalee (Illinois State Museum), and W. D. Turnbull (Field Museum of Natural History) for critically reviewing the manuscript and for providing helpful information. Drs. Parmalee and Turnbull also made available for study specimens in their care. Drs. M. S. Peterson (American University, Washington, D.C.) and F. C. Whitmore, Jr. (U. S. Geological Survey) aided in the initiation of this study. Dr. D. M. Moore (Knox College) carried out the X-ray analysis of the matrix associated with the *Ovibos* cranium from Henderson County. Figure 1 was prepared by Mr. Lawrence B. Isham, scientific illustrator for the Department of Paleobiology, U. S. National Museum.

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Manuscript received Nov. 10, 1967.

THE EFFECTS OF STAINING WITH BUFFERED AZURE A EOSIN B AFTER FIXATIVES CONTAINING PICRIC ACID

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ABSTRACT. — In tissues fixed in fixatives containing picric acid and stained with the azure A eosin B technic, the nuclei were stained either with acid dye or with basic dye depending upon the pH of the staining solution.

During routine staining with the buffered azure A eosin B method (Lillie 1965), it was observed that the nuclei of tissue fixed in modified Bouin's fluid (Lillie 1965) stained pink with eosin B (acid dye) at pH 4.0 and stained blue with azure A (basic dye) at pH 6.5.

An investigation of this problem was conducted, and the results are presented in this paper.

METHODS AND MATERIALS

Tissue from a human skin tumor and from a guinea pig spinal cord were fixed in each of the following fixatives: modified Bouin, picro-formalin (10% formalin in saturated aqueous picric acid), picro-acetic acid (5% glacial acetic acid in saturated aqueous picric acid), and in saturated aqueous picric acid. The control tissues were fixed in 10% formalin.

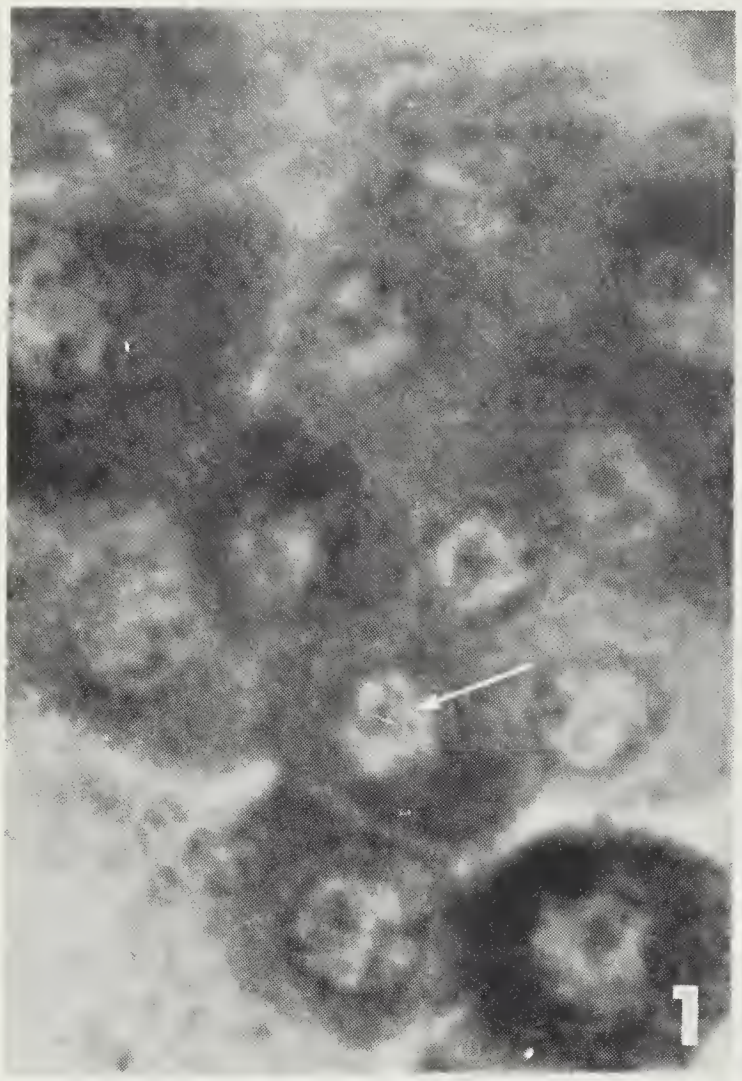
All tissues were then embedded in paraffin by the standard procedure and sectioned at 6μ.

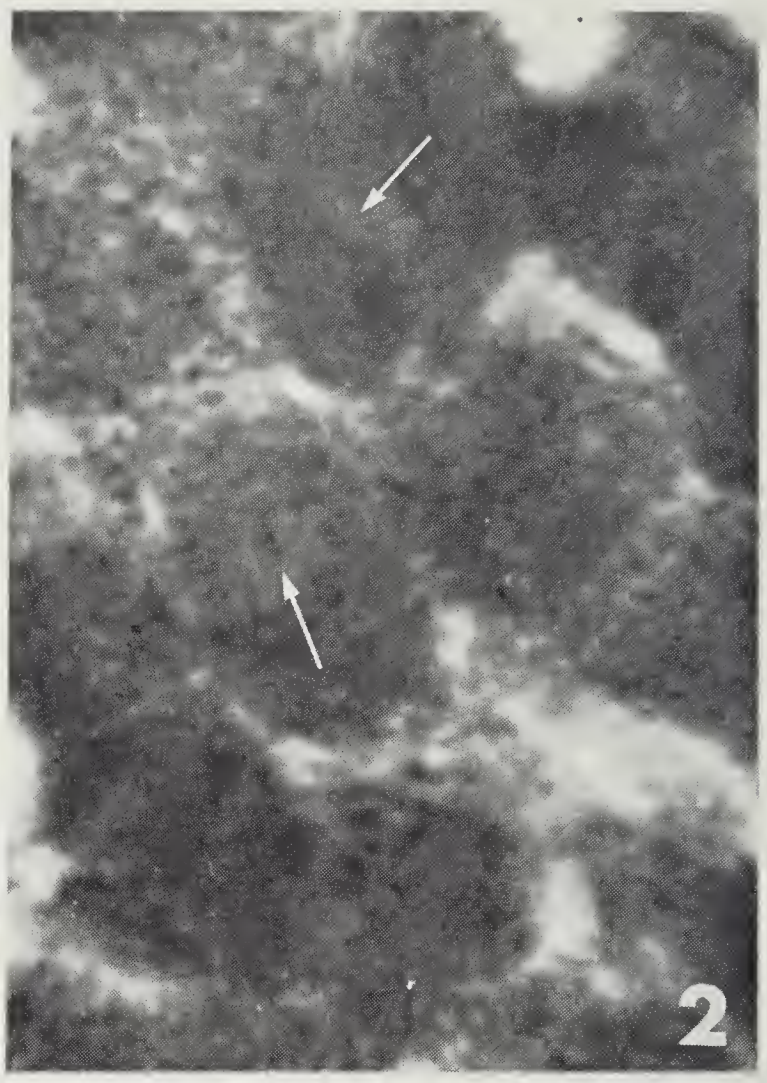
Staining with azure A eosin B was done in a stepwise series of staining mixtures graduated by pH 0.5 from pH 4.0 to pH 7.5. Staining solutions were buffered with Walpole's acetate buffer from pH 4.0 to pH 5.5 and with Sorensen's phosphate buffer from pH 6.0 to pH 7.5 (Lillie 1965). The final dilutions were 0.01 M for acetate buffers and 0.0125 M for phosphate buffers. As a control, two series of staining mixtures comparable to that above were set up in which eosin B was replaced with the same amount of Fast green FSH or Biebrich scarlet.

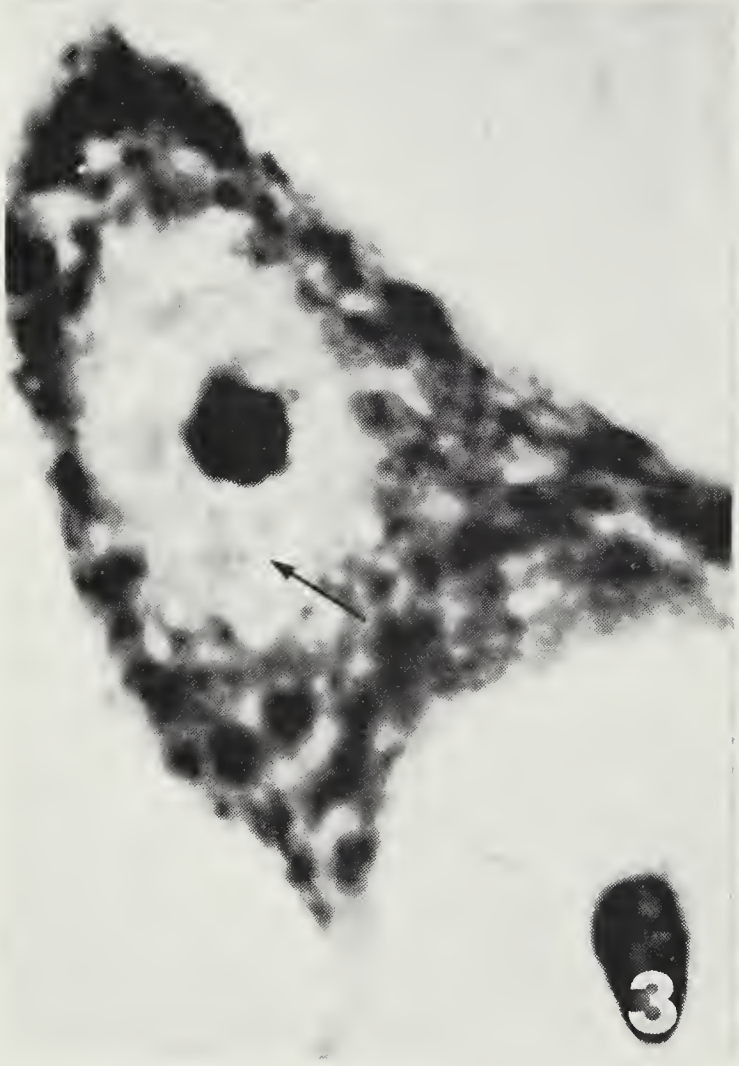
RESULTS

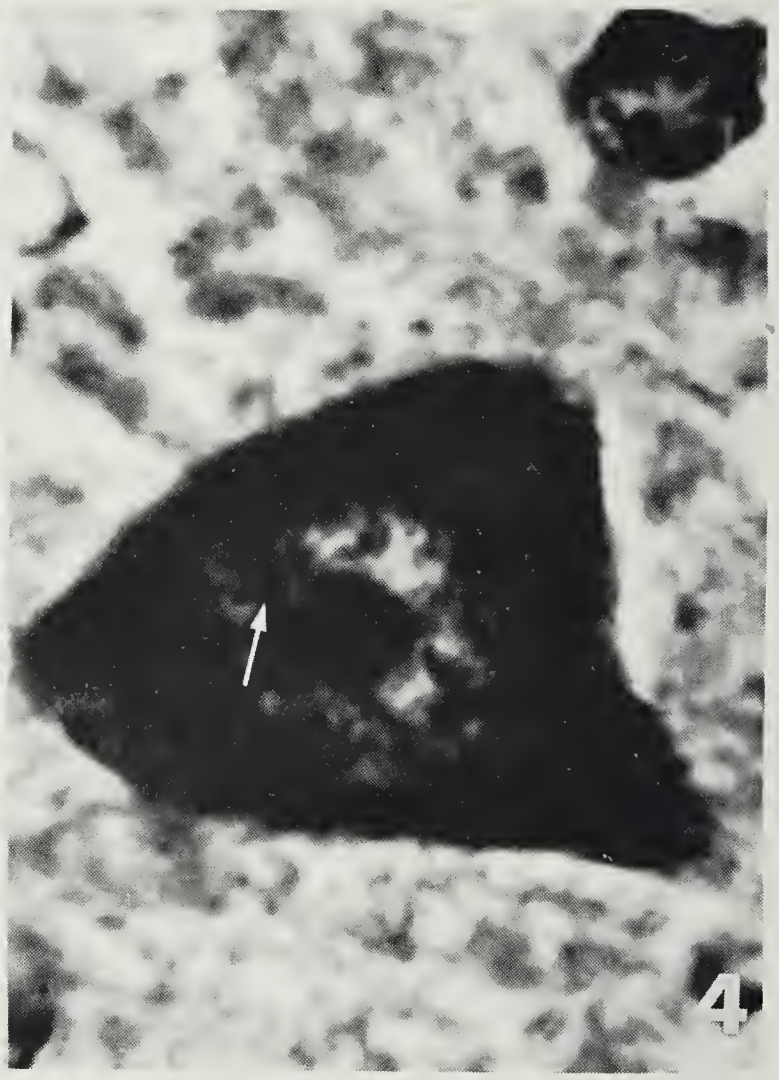
The staining results were similar for tissues fixed in fixatives containing picric acid. The morphological details were best preserved in tissue fixed in modified Bouin's fluid. At pH 4.5 and below, the nuclei were stained with the acid dye (Figures 1 and 3). At pH 6.0 and above, the nuclei were stained with the basic dye (Figure 4). Between these limits, the nuclei were stained with both dyes. Nuclei of the control tissue fixed in 10% formalin were stained with the basic dye at all pH's used (Figure 2).

The type of acid dye used (eosin B, Fast green FSH, or Biebrich scarlet) did not influence the results. The cytoplasmic basophilia and nucleoli were stained with the basic dye regardless of the fixative or pH used (Figures 1, 2, 3, and 4).









FIGURES 1-4. All preparations were stained with the buffered azure A eosin B technic. X 1,800.

1. — Cells of the human skin tumor, fixed in modified Bouin, and stained at pH 4.0. Arrow points to the nucleus.

2. — Comparable tissue, fixed in 10% Formalin, and stained at pH 4.0. Arrows point to nuclei. Compare with figure 1.

3. — Neuron from guinea pig spinal cord, fixed in modified Bouin, and stained at pH 4.5. Arrow points to the nucleus.

4. — Same tissue but stained at pH 6.5. Arrow points to the nucleus. Compare with figure 3.

Discussion

Some fixatives will permanently increase the affinity of tissue proteins for either basic or acid dyes. Tolstoouhov (1928) showed that this phenomenon was due to a permanent alternation of the isoelectric point of these proteins, toward the lower or higher pH value, caused by the particular fixative fluid. Fixatives containing picric acid render the cytoplasmic proteins acidophilic and produce unusual staining results with nucleic acids. Spicer (1961) and Lhotka and Davenport (1947) showed that fixation with Bouin's fluid produced aldehyde groups in DNA (deoxyribonucleic acid). Lillie (1965) reported that picric acid rendered RNA (ribonucleic acid) insoluble and more resistant to digestion with ribonuclease. Baker (1958) stated that picric acid separated protein of the chromatin from DNA, which was then dissolved.

Our results are in agreement with the last two statements. The dissolved DNA did not stain in tissues fixed in fixatives containing picric acid. The coagulated protein of the chromatin stained either with acid dye (pink) or basic dye (blue) or both (purple) according to the pH of the staining fluid (Baker 1958, Singer 1952). Our results may be explained in a different way. The reactive groups of DNA were either masked with picric acid or firmly bound to the protein of the chromatin. The staining reaction of RNA with basic dye was not affected by the pH of the staining solution or by fixation. Lillie's buffered azure A-eosin B technic used at pH 4.0 or 4.5 for tissue treated with a fixative containing picric acid offers striking staining contrast in cells with strongly basophilic cytoplasm.

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Manuscript received Oct. 19, 1967.

EFFECT OF COLD EXPOSURE ON RESPIRATORY ACTIVITY OF RAT LIVER HOMOGENATES AND MITOCHONDRIA

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ABSTRACT. — The effect of cold exposure (3-7 weeks at 5C) on respiratory activity of rat liver mitochondria and liver homogenates was measured polarographically at 5, 15, 25 and 35 C using the YSI oxygen monitor system. Mitochondrial respiration rates (using succinate and beta-hydroxybutyrate in the presence and absence of nucleotides), as well as the efficiency of oxidative phosphorylation, proved to be identical in the cold-exposed and control rats at all four incubation temperatures. In homogenates, however, the succinate-ADP respiration rate was higher in experimentals than controls at 25 C and 35 C, whereas β-hydroxybutyrate activity was not affected by cold exposure. An Arrhenius plot of these data indicated a higher energy of activation for the succinoxidase, but not the β-hydroxybutyrase, reaction in cold-exposed animals. The significance of these findings is discussed in relation to previous studies based on respiration rate measurements at only one incubation temperature.

There have been numerous attempts to define the precise cellular enzymatic mechanisms involved in cold acclimation of rats. At present two mechanisms are offered to explain how mitochondria might facilitate increased tissue thermogenesis and thereby provide rats with greater capability to produce heat during cold exposure. One involves the partial uncoupling of oxidative phosphorylation thus channeling more energy from substrate oxidation directly into heat (Smith and Fairhurst, 1958). The other entails a generalized increase in the rate of tissue oxygen consumption as the major source of body heat (Frehn and Anthony, 1965).

It is safe to state, however, that to date available data on cellular metabolic events which accompany cold acclimation are incomplete and contradictory (Frehn and Anthony, 1965; Aldridge and Stoner, 1963; Chaffee *et al.*, 1961; Frehn and Anthony, 1962; Patkin and Masoro, 1960; Horwitz, 1967). This stems, in part, from procedural differences in tissue preparations used (slices, homogenates, mitochondria), in methods of analysis, and in temperatures at which measurements were made.

The present study involved a comparison of selected aspects of respiratory metabolism in liver mitochondrial and homogenate preparations from cold-exposed rats. All measures were performed at 5, 15, 25 and 35 C to determine whether adaptive metabolic alterations are temperature dependent. One of the aims of this work was to establish the possible

importance of extramitochondrial components in cellular adaptation during cold exposure.

Materials and Methods

Twenty-four male rats (150-200g, Holtzman) were exposed to 5 C for 3-7 weeks, with 24 controls maintained at 24 ± 1 C; these were used for measures of liver mitochondrial respiration. A second group of 18 cold-exposed rats and 18 ambient temperature controls was used in liver homogenate respiration studies. Food (Purina laboratory chow) and water were supplied *ad libitum*. All animals were on an 8 hour daily light regimen and were housed individually in stainless steel cages containing bedding.

Initial steps in tissue preparation were carried out at 1-2 C. Animals were killed by cervical fracture, livers quickly removed and placed in ice-cold isolation medium (225 mM mannitol, 75 mM sucrose, 0.1 mM EDTA). The homogenates were prepared by grinding with a Potter-Elvehjem homogenizer by forcing the rotating Teflon pestle to the bottom of the vessel six to eight times. The tissue suspension was then strained through cheesecloth to remove extraneous matter. Mitochondria were prepared according to Schneider method (Schneider, 1948), washed, and the whole liver mitochondrial pellet suspended in 1 ml of isolation medium. The tissue preparations were kept cold until addition to the reaction vessel.

Respiration and phosphorylation measurements were made polarographically using a YSI oxygen monitor system. Respiration rates, measured over a period of 3-5 minutes, were expressed as micro-molar oxygen consumed per second per milligram of nitrogen. This form of expressing respiration rates has been described by Chance 1959). The reaction medium contained 45 mM mannitol, 15 mM sucrose, 40 mM KC1, 20 mM $MgCl_2$, 20 mM potassium phosphate buffer (pH 7.3), and 0.02 mM EDTA. Each reaction vessel contained 3 ml of reaction medium. Mitochondrial and homogenate respiration rates were measured with exogenous substrate alone (10 mM succinate or β-hydroxybutyrate), with substrate plus ATP (0.02 ml of 0.1 M, homogenates only), and with substrate plus ADP (0.01 ml of 0.05 M). The concentration of the stock solution of ADP was determined spectrophotometrically at 259 mμ. ATPase activity of homogenates was determined by measuring the rate of homogenate respiration in the presence of excess substrate and ATP and noting the respiratory stimulation caused by the addition of the ATP. It has been found that ATP stimulates respiration in homogenates (Ziegler et al., 1963), but not in mitochondria (Chance and Williams, 1956; Chance, 1961). Previous kinetic data leave little doubt that the action of ATP on homogenate respiration is dependent on ATPase activity (Ziegler et al., 1963).

ADP addition speeds up respiration of both mitochondria and homogenates several-fold to give the maximum possible respiration. The ratio of substrate-ADP respiration to substrate respiration is called the respiratory stimulation (Rs) caused by ADP addition. The P:O ratio was obtained by measuring the oxygen consumed during phosphorylation of a known amount of added ADP. This value provides a measure of the efficiency of oxidative phosphorylation. Nitrogen content of mitochondrial and homogenate suspensions (mg N/ml) was determined by the micro-Kjeldahl method (Ma and Zuazaga, 1942).

Results

Data on the effect of cold exposure on P:O ratios in mitochondria are shown in Fig. 1. In the presence of succinate the P:O ratio proved to be identical in liver mitochondria of cold-exposed and control rats at all four temperatures. With β-hydroxybutyrate the mean P:O values were slightly lower in cold-exposed rats, but not significantly so. Both controls and cold-exposed groups exhibited a reduction in efficiency of oxidative phosphorylation at 35 C. Thus, these findings indicate that when washed mitochondrial preparations are used no appreciable alteration in oxidative phosphorylation is evident in cold-exposed rats.

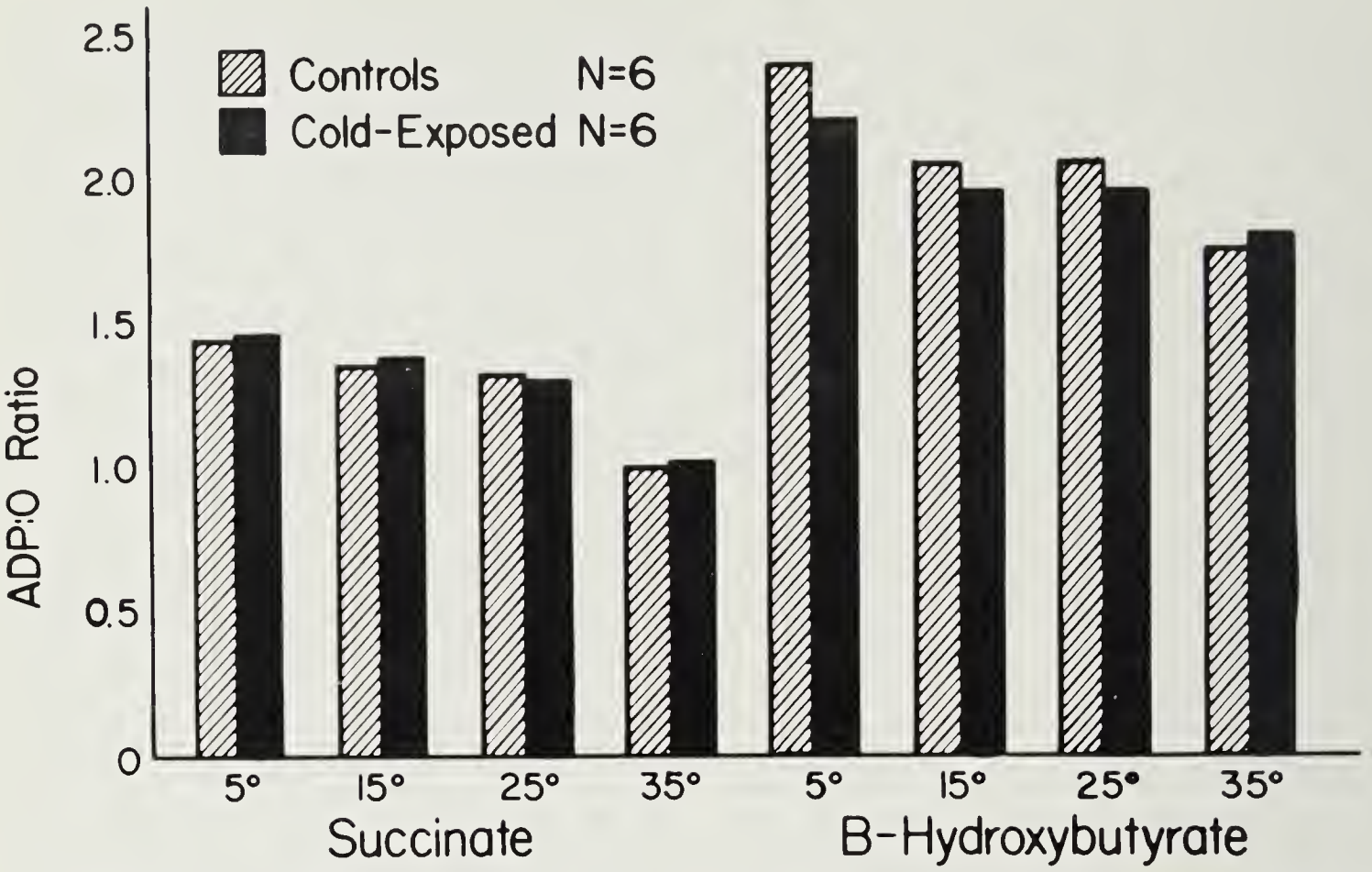

FIGURE 1. — Effect of cold (5 C) for 3-7 weeks on oxidative phosphorylation in rat liver mitochondria.

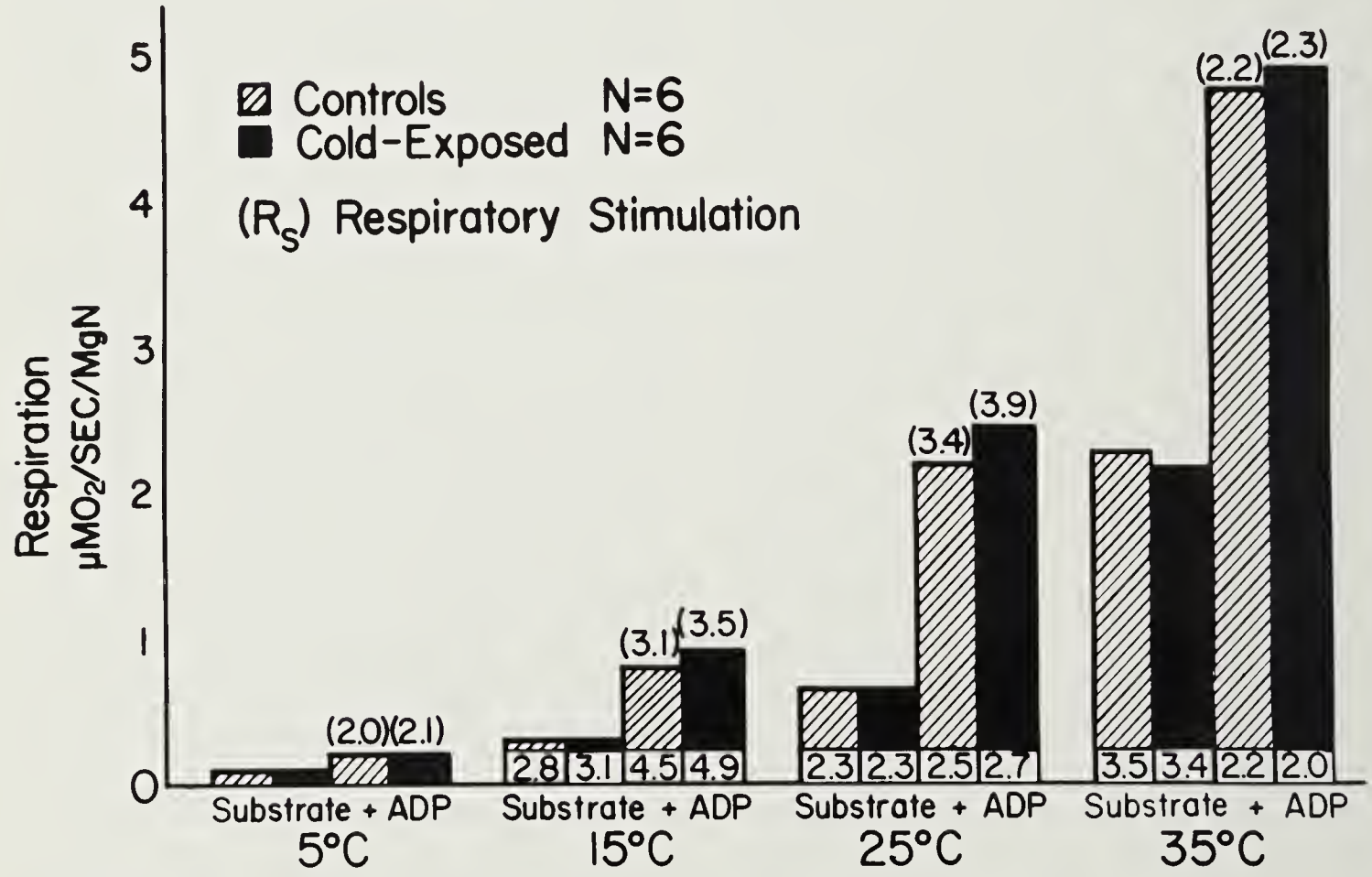

FIGURE 2. — Effect of cold (5 C) for 3-7 weeks on succinate respiration in rat liver mitochondria. R_s values, representing the respiratory stimulation caused by adding ADP, are given in parentheses. Q_{10} values are included at the base of the bars.

In addition to phosphorylation measurements, respiratory rates were also determined on the same mitochondrial suspensions. Respiratory rates, expressed in μM O_2/sec/mg nitrogen, for the two substrates, succinate and β-hydroxybutyrate, before and after the addition of excess ADP are summarized in Figures 2 and 3. No differences in mitochondrial respiratory activity were observed between control and cold-exposed animals. At all temperatures studied, ADP stimulated substrate respiration to the same extent in cold-exposed and control animals. The highest Q_{10} values, with both substrates, were obtained between 5 and 15 C with ADP present in excess. Thus, again when washed mitochondria are used, the data indicate that cold-exposed and control rats exhibit no differences in succinoxidase activity, β-hydroxybutyrase activity, or respiratory stimulation with ADP.

The results using rat liver homogenates are shown in Figures 4 and 5. Measurements of the effects of succinate, β-hydroxybutyrate, ATP and ADP are included for only three temperatures since rates at 5 C were too slow to measure accurately. At all temperatures studied, homogenate respiration in the presence of excess substrate alone proved to be unaffected by cold exposure. Likewise, exposure to cold did not cause an appreciable alteration in the ATPase activity of the liver tissue at 15 and 25 C. At 35 C, however, the ATPase levels tended to be slightly increased in cold-exposed groups as evident from the higher substrate-ATP respiration rates ($P<0.05$).

It is also apparent in Figures 4 and 5 that the addition of ADP to tissue homogenates stimulates succinate and β-hydroxybutyrate respiration rates at 35 C to a greater extent in cold-exposed animals than

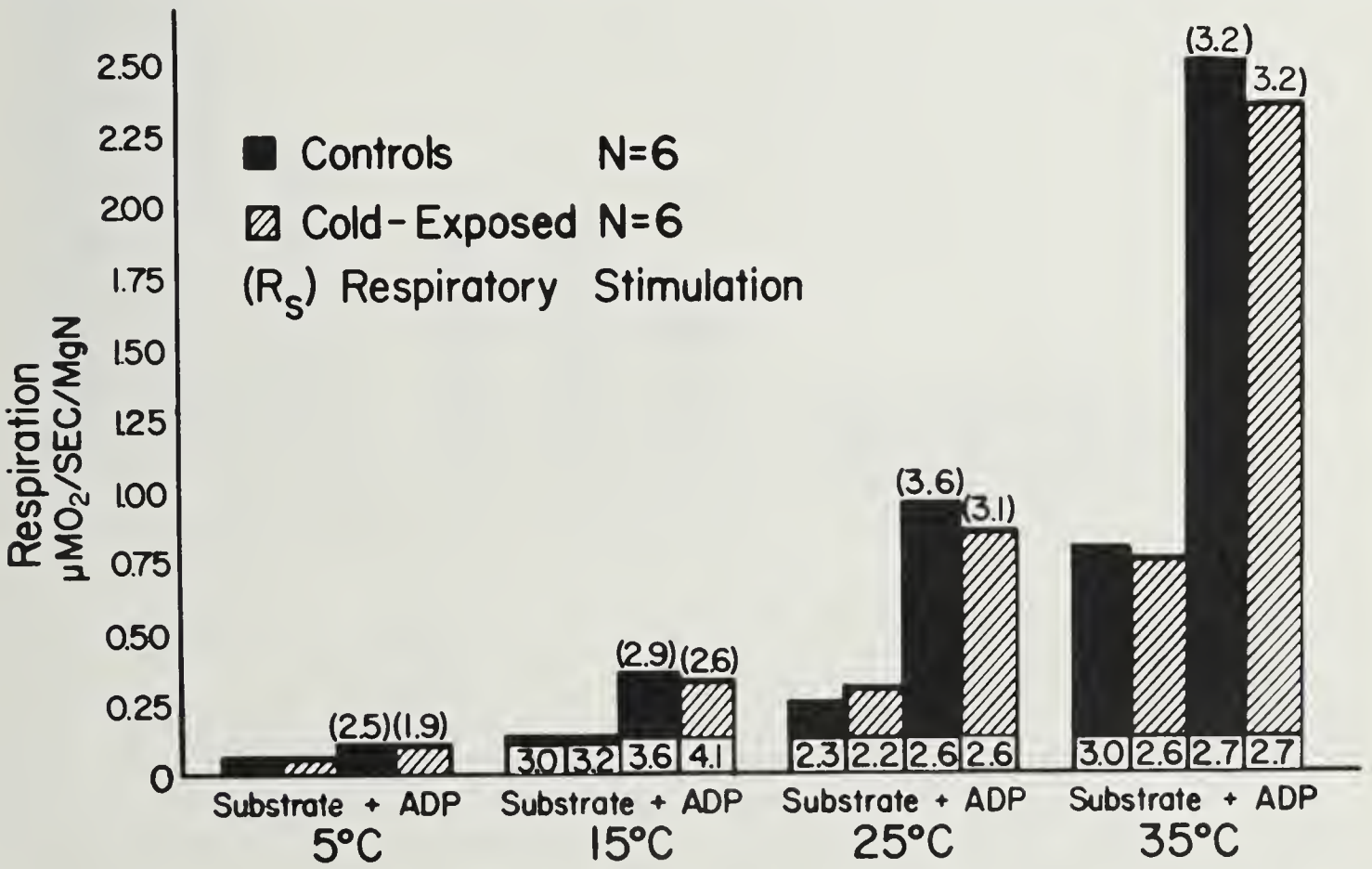

FIGURE 3. — Effect of cold (5 C) for 3-7 weeks on β-hydroxybutyrate respiration in rat liver mitochondria.

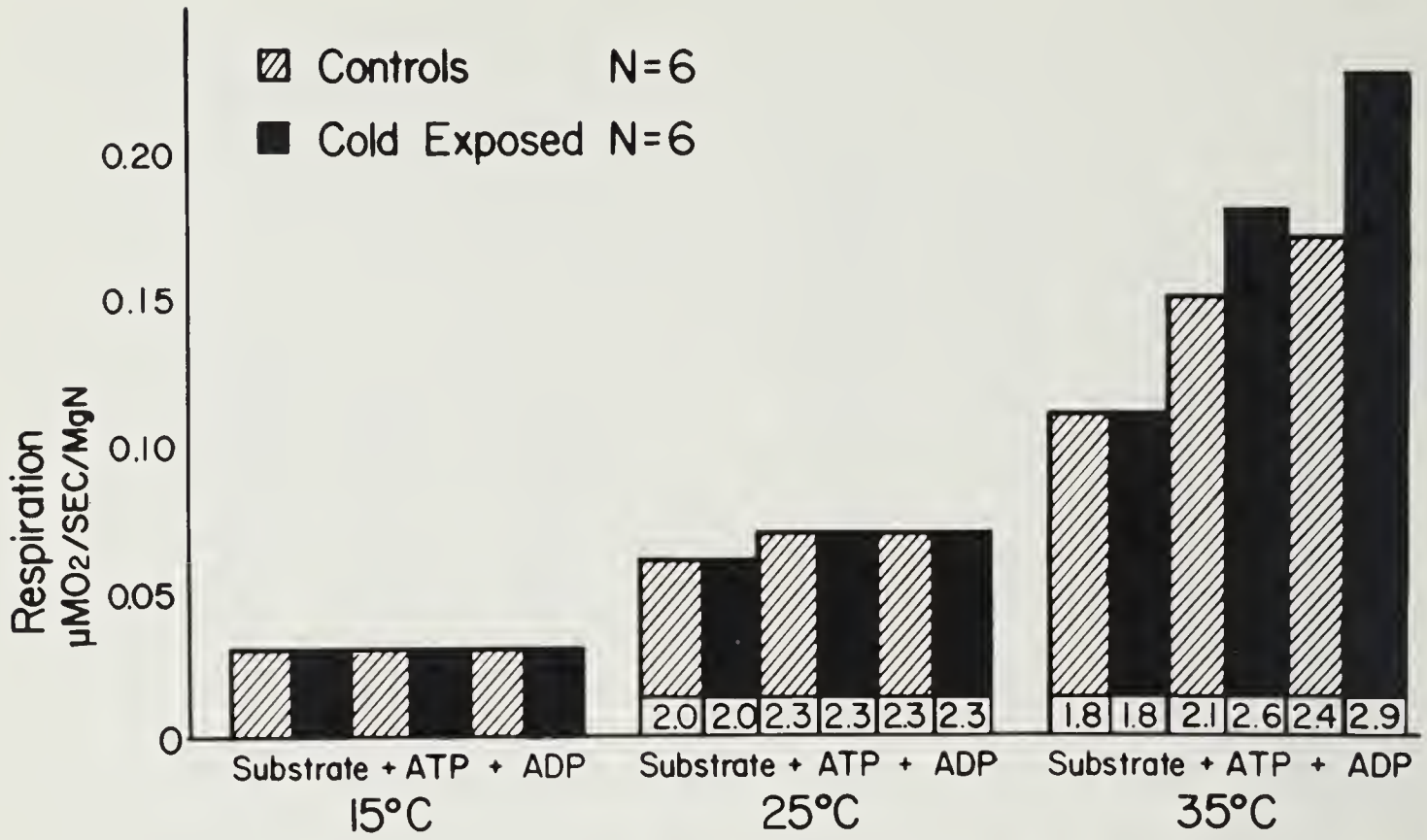

FIGURE 4. — Effect of cold (5 C) for 3-7 weeks on β-hydroxybutyrate respiration in rat liver homogenates.

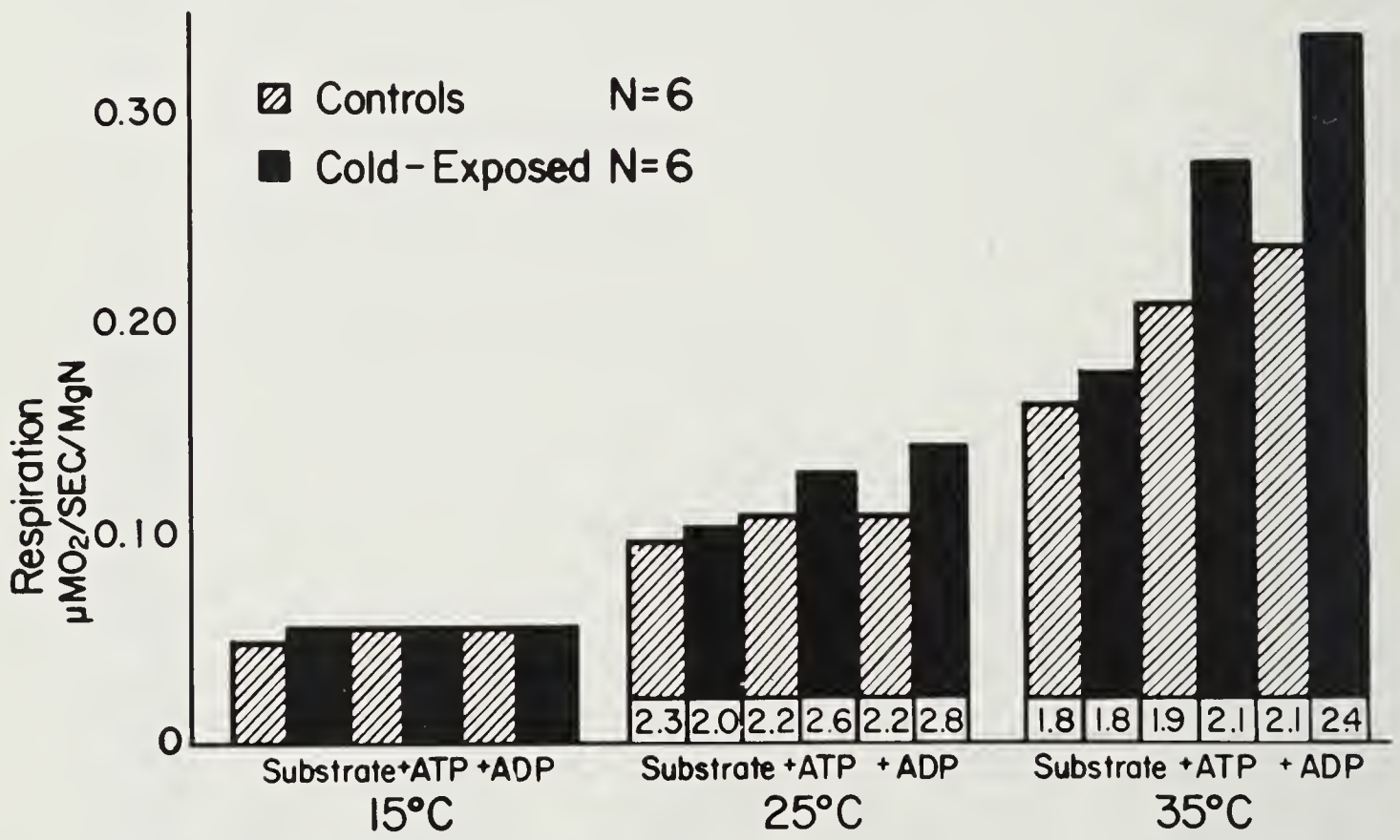

FIGURE 5.—Effect of cold (5 C) for 3-7 weeks on succinate respiration in rat liver homogenates.

it does in controls ($P<0.01$). Thus, the maximum rate of tissue homogenate respiration (i.e., respiration in the presence of excess substrate plus ADP) is significantly greater in cold-exposed rats ($P<0.05$). This increase is in contrast to the unaltered succinate-ADP respiration observed with washed mitochondria. Further examination of the data in

Fig. 5 suggests that the tissue succinate-ADP respiration rate is exhibiting a differential temperature response. Thus, by analyzing the succinate-ADP rate of respiration at the various temperatures in both the control and cold-exposed groups, one sees that at 15 C they are identical, at 25 C the cold-exposed value is increased by 22% above the control rate, and at 35 C the cold-exposed rate is increased by 44% above control. This is also evidenced from the observation that with homogenates the Q_{10} values of substrate-ADP respiration tended to be slightly higher in cold-exposed rat homogenates than in controls.

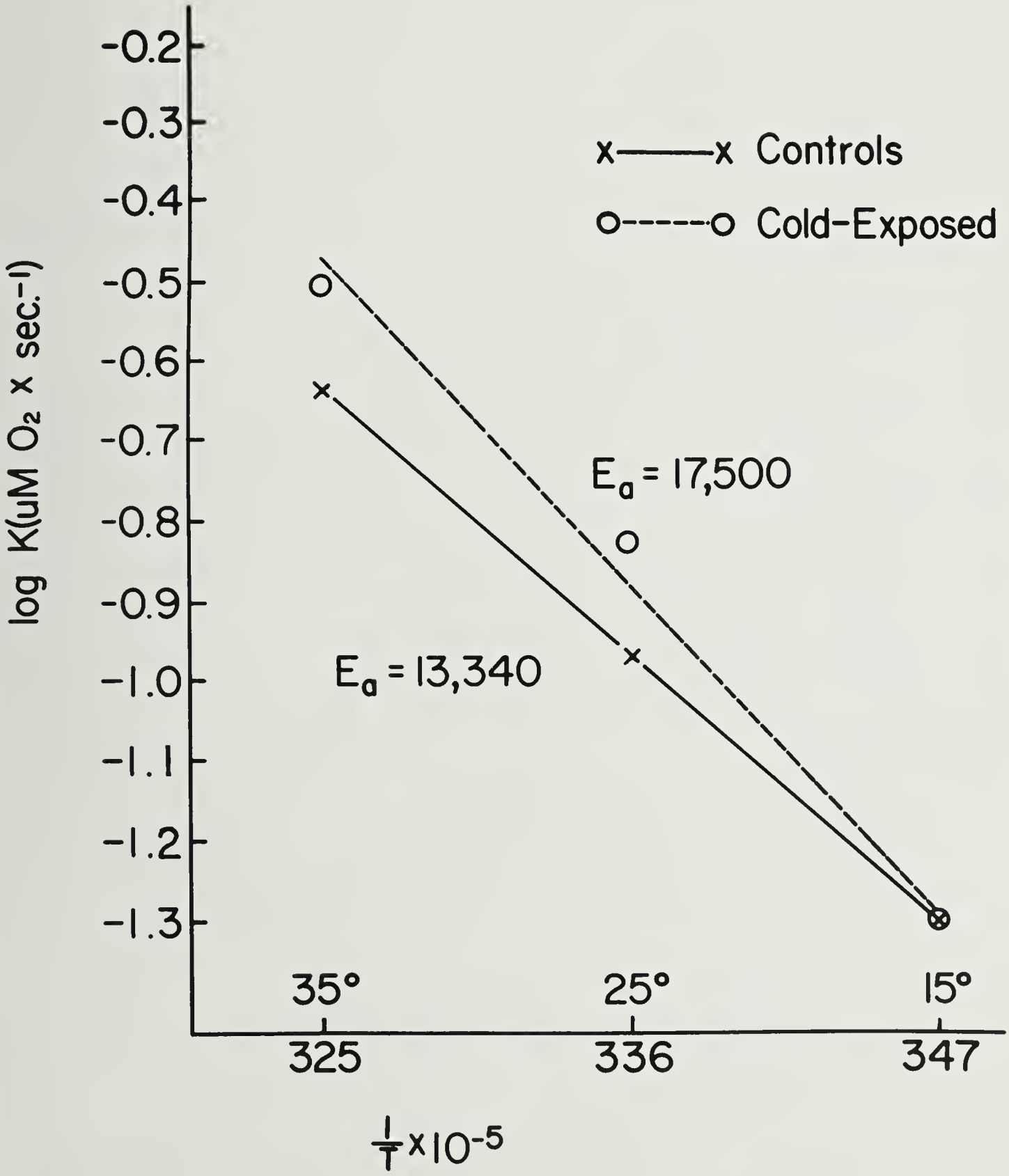

FIGURE 6. — Arrhenius plot of data on succinate-ADP respiration rates of liver homogenates from control and cold-exposed rats.

An alternative way of comparing the effects of temperature on the substrate-ADP respiration rates is to express the data in the form of an Arrhenius plot. In this way one can calculate the energy of activation (E_a) which is the slope of the line obtained in plotting log K (respiration rate in $\mu M\ O_2 \times sec^{-1}$) against the reciprocal of the absolute temperature. From Figures 6 and 7 it is apparent that E_a values of the succinoxidase and β-hydroxybutyrase reactions in the presence of excess ADP were essentially the same over the 15 to 35 C temperature range in homogenate preparations from cold-exposed and control rats. How-

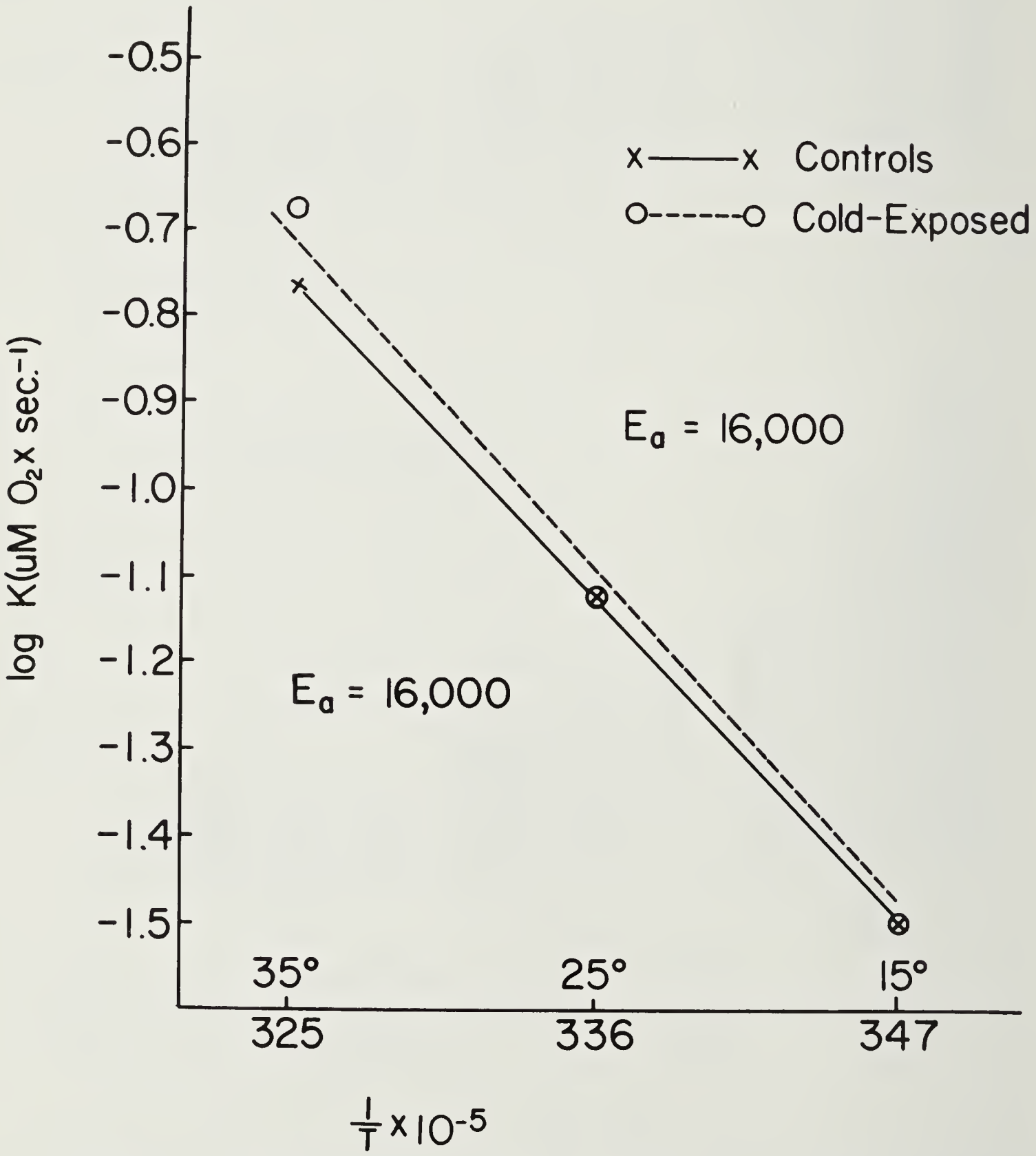

FIGURE 7. — Arrhenius plot of data on β-hydroxybutyrate-ADP respiration rates of liver homogenates from control and cold-exposed rats.

ever, if one considers only the 25 to 35 C interval, which more closely corresponds to the animal's own temperature, the E_a for the succinoxidase reaction appears higher in cold-exposed versus the control group. Plotting the data obtained from mitochondrial preparations in a similar fashion indicated there were no differences observed in the E_a of succinate- or β-hydroxybutyrate - ADP respiration rates between control and experimental groups (Fig. 8). It is also evident from Fig. 8 that the data fit a straight line over the temperate range from 15 to 35 C but that the line drops off between 15 and 5 C.

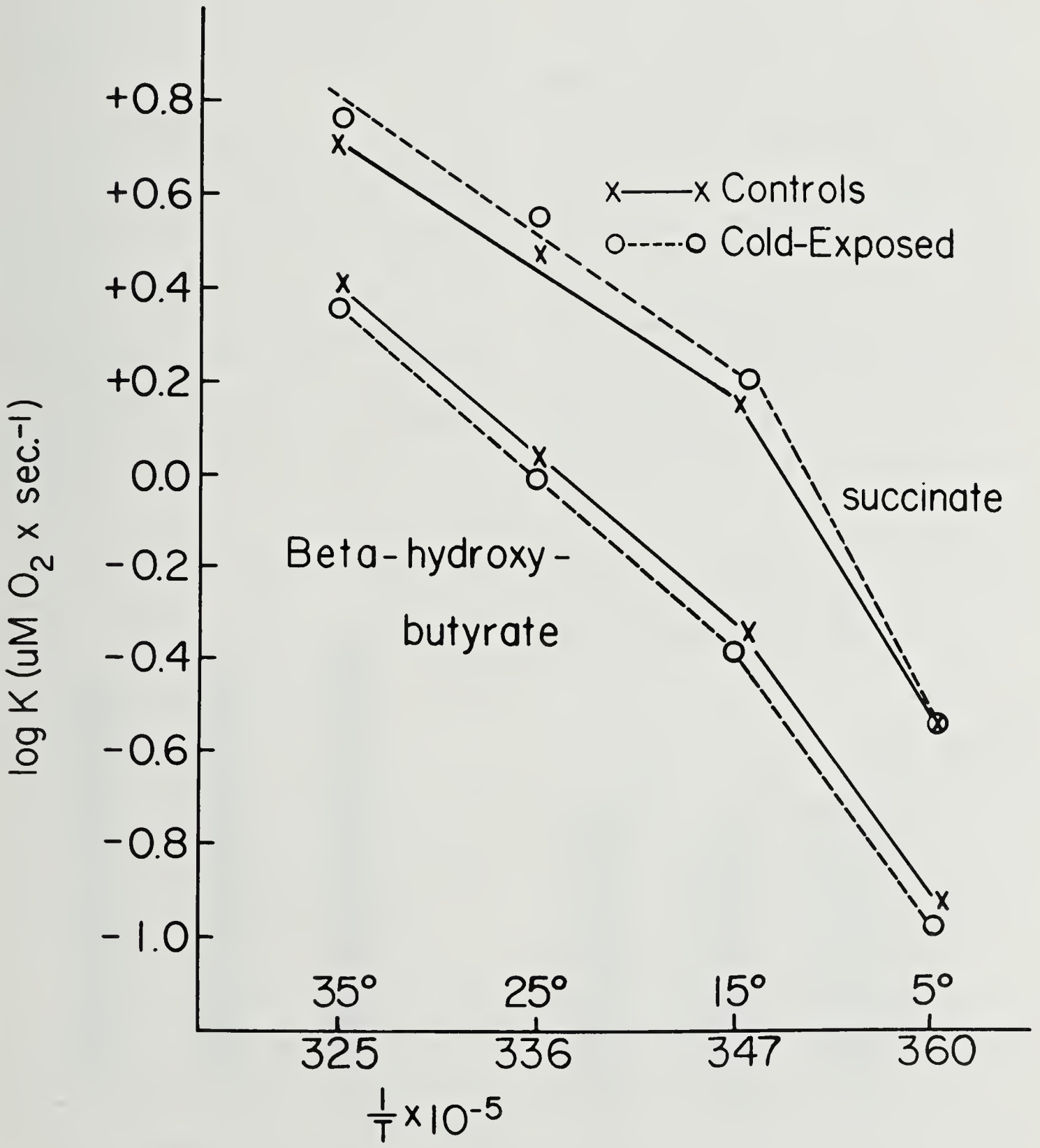

FIGURE 8. — Arrhenius plot of data on substrate-ADP respiration rates of liver mitochondria from control and cold-exposed rats.

The P:O ratios obtained with liver homogenates are shown in Fig. 9. In the presence of both succinate and β-hydroxybutyrate, the P:O ratios were significantly reduced ($P<0.05$) in sold-exposed rats relative to controls. Furthermore, the extent of reduction was the same at all three temperatures used.

Discussion

The findings in this study using both liver homogenates and mitochondria agree with several earlier reports that an increased respiration rate can be demonstrated if one used liver slices or homogenates from cold-exposed rats (Clark *et al.*, 1954; Hannon, 1960; Hannon, 1958). The data presented also are consistent with those reports which claim an absence of cold-induced alterations when measurements are made using washed mitochondrial preparations (Chaffee *et al.*, 1961; Patkin and Masoro, 1960). Thus, our results add support to the belief that increased tissue respiration observed during cold exposure is probably influenced by some extra-mitochondrial factors which are lost in washed mitochondrial preparations (Smith, 1960; Smith and Hoijer, 1962). We as yet have no knowledge of what this influence may be or how it works.

One of the most important findings of this investigation is that the only instance where a difference was found between cold-exposed and control rats was with the succinate-ADP respiration rate of homogenates at 25-35C. Since the various metabolic states of mitochondrial respiration are known to be influenced by a number of factors, respiratory rates measured using different substrates

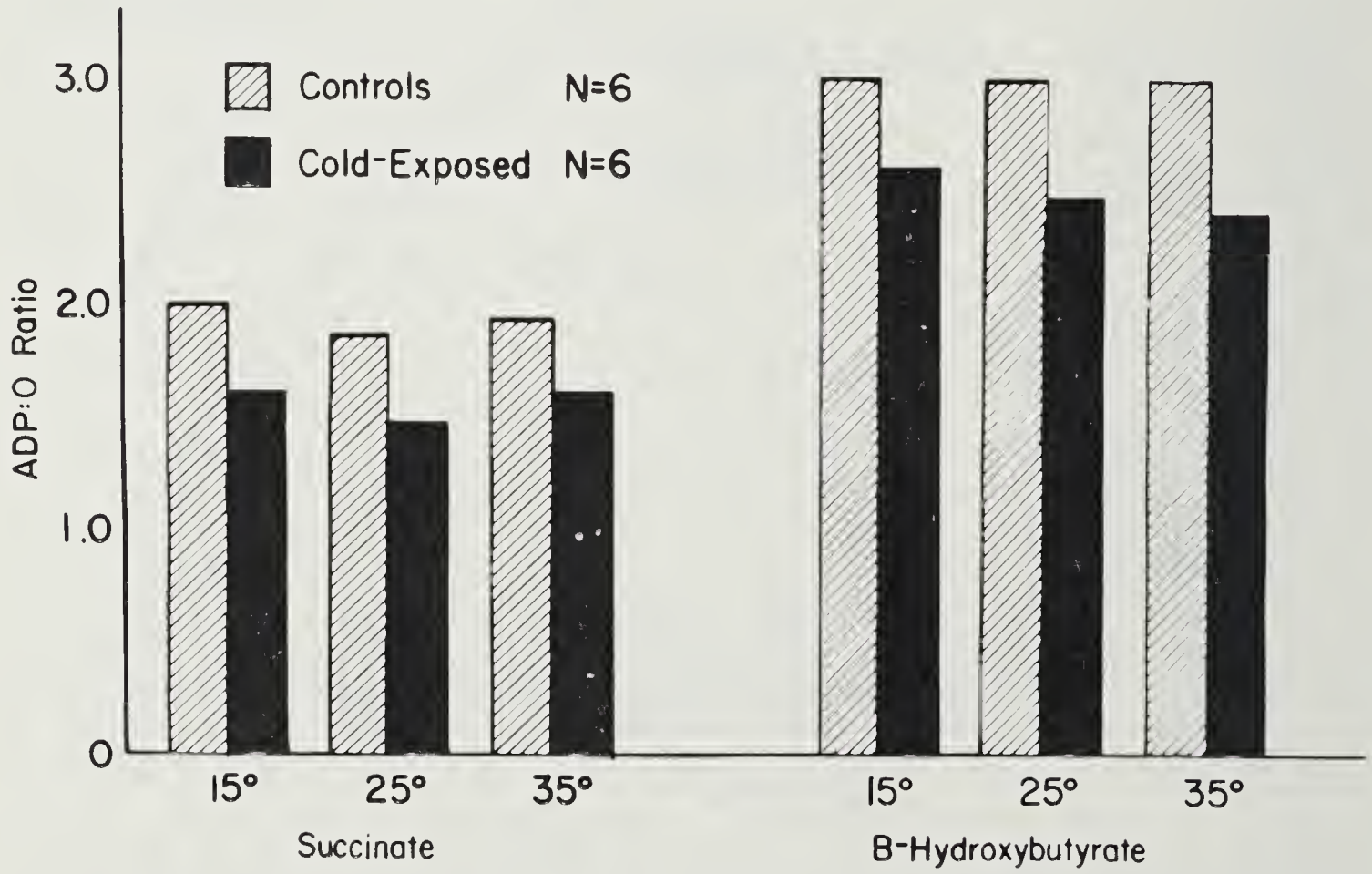

Figure 9. — Effect of cold (5 C) for 3-7 weeks on oxidative phosphorylation in rat liver homogenates.

and nucleotides add to our understanding of the regulatory mechanisms involved in oxidative metabolism. For example, substrate respiration may be affected by such factors as endogenous ADP production, changes in oxidative phosphorylation or large changes in dehydrogenase activity (Smith and Fairhurst, 1959). The absence of any alteration in succinoxidase or β-hydroxybutyrase activity in homogenate respiration found in this study indicates that none of these regulatory mechanisms is affected by cold exposure. Similarly, the ratio of the substrate-ATP respiration rate to the substrate respiration rate can be used as a measure of ATPase activity of homogenates. Thus, the observation that ATP stimulated succinate and β-hydroxybutyrate homogenate respiration to the same extent, at all temperatures, in control and cold-exposed rats indicates that cold exposure does not cause any appreciable alteration in the ATPase activity of liver tissue. These results are in agreement with previous reports that ATPase activity does not change during cold exposure. (Hannon, 1959).

Chance *et al.* (1955) have shown that the substrate-ADP respiration rate is a reliable measure of the concentration of functional respiratory chain units in mitochondria. In a preliminary study, we observed an increased substrate-ADP respiration rate (at 25 C) in liver homogenates from cold-exposed rats and concluded that an increased concentration of respiratory enzymes constituted an important aspect of cold acclimation (Frehn and Anthony, 1965). If this adaptive cellular response was the only factor operating to bring about increased oxygen consumption during cold exposure, one would expect to demonstrate an increased substrate-ADP respiration rate with both homogenates and mitochondrial preparations. This did not prove to be the case. Cold exposure produced no detectable changes in oxidative metabolism in mitochondrial preparations. On the other hand, studies using homogenates showed differences in both succinate-ADP respiration rates and in oxidative phosphorylation attributable to cold exposure.

On the basis of Arrhenius plots of substrate-ADP respiration rate data, it was tentatively concluded that the E_a of the succinoxidase reaction was higher in liver homogenate of cold-exposed rats than of controls. It would be desirable, however, to repeat this phase of work at several additional temperature increments between 25 and 35 C to establish the extent to which this constitutes an important adaptive response. An increased E_a of the succinoxidase-ADP reaction could be indicative of an increased energy requirement in this step of carbohydrate oxidation and perhaps signify a shift toward other reactions involving fat metabolism during cold exposure (since the E_a of the β-hydroxybutyrase reaction was not altered). This would be in keeping with the concept that cold-acclimated animals exhibit an increased capacity to oxidize long-chain fatty acids (Felts and Masoro, 1959). The observation that the E_a of this reaction is not altered in mitochondrial preparations in cold-exposed rats again emphasizes the difference in respiratory regulation be-

tween homogenates and mitochondria. In addition, the finding of an apparent break in the Arrhenius plots of mitochondrial respiration at approximately 15 C with both substrates bears further study, particularly in hibernating animals where the body temperature actually drops below this level.

Since the P:O value indicates the extent to which phosphorylation is coupled to respiration, this ratio provides a measure of the efficiency of oxidative phosphorylation. Thus, a decrease in P:O ratio in mitochondria from cold-exposed animals would indicate that more of the available energy was being converted into heat and less into stored energy in the form of ATP. The results of this study failed to show any decline in the P:O ratios in mitochondria but did show a decreased P:O ratio in homogenates from cold-exposed rats. It is probable that extra-mitochondrial factors which, it has been suggested, influence respiration during cold exposure also influence oxidative phosphorylation. Therefore, it seems reasonable to assume that both the oxidative and phosphorylative mitochondrial functions may contribute to the "non-shivering thermogenesis" frequently observed in cold-exposed rats.

Acknowledgments

This research was supported by Public Health Service Research Grants GM-13358 and GM-07678, from the Division of General Medical Sciences.

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Manuscript received Nov. 27, 1967.

NOTES

BACULUM DEVELOPMENT IN MINK

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ABSTRACT. — Data are presented on baculum growth in mink. Difficulties associated with determining age by baculum morphology are indicated.

Wright (1947, 1951) and Deanesly (1935) have shown the changes in the size and shape of the baculum in the long-tailed weasel (*Mustela frenata*) and ermine (*Mustela erminea*) are related to increased levels of the male sex hormone. Mature baculum form is attained during the first breeding season (January through March, depending on local weather conditions, Trippensee, 1953), shortly before the males are one year old. Since differences in baculum conformation are reportedly reliable for separating young of year from adult males (Elder, 1951; and Lechleitner, 1954), the present report concerns difficulties associated with determining age during the critical period of the first breeding season.

In this study, bacula from a series of 68 wild-trapped mink, *Mustela vision mink*, taken during November through February 1961, 1962 and 1963 in Wake and Forsyth counties, North Carolina, all showed a similar growth pattern. All bacula were cleaned by dermestid beetles, weighed, and measured (total length). An attempt was made to distinguish young-of-year from individuals older than one year using established techniques. Length and width measurements of testis were recorded.

Testis size averages (Table 1) indicated a rapid testicular enlargement in November and December and little if any further enlargement from that time through the end of February. This would indicate the males are probably ready to breed by early January. Average weight of the bacula collected increased beginning in mid to late December and continued until mid-February (Table 1). This corresponds to the baculum growth cited above by Wright and Deanesly for the long-tailed weasel and ermine.

Of 41 bacula removed from specimens trapped in November and December, 14 could be classified as adult types and 27 as young-of-year types. The remaining 27 bacula taken in January, February, and March were morphologically adult but several were smaller in length and lighter in weight than adult bacula in the November and December series. Both Elder (1951) and Lechleitner (1954) indicated baculum weights were

TABLE 1.—Seasonal differences in average testis size (length plus width in mm) and average baculum weight (mg) of North Carolina mink.

Date	Nov.	Dec. 1-15	Dec. 16-31	Jan. 1-15	Jan. 16-31	Feb.
Number of Specimens	16	14	11	9	7	11
Testis	20	32	39	40	39	38
Baculum	190	210	250	290	340	350

not suitable for separating young-of-year from adults at the 95 per cent level, yet both indicated baculum weight probably could distinguish these age groups with 80 to 90 per cent accuracy. These smaller adult-type bacula from the present study are believed to be those of young-of-year animals that had only recently reached reproductive maturity. Attainment of the adult bacular conformation thus seems to precede attainment of full adult bacular length and weight, and apparently occurs when the male mink is approximately 10 months old. These smaller bacula (from January and February) compared with bacula taken in November and December, would be classified as intermediate between young-of-year and adult type based on weights.

Close examination of the finds of Elder, 1951, Lechleitner, 1954, and Greer, 1957, reveals that their age criteria determinations were largely based on studies of known-age (pen-raised) mink ½, 1½, 2½, etc., years of age. The reliability of baculum morphology in distinguishing young-of-year from adult animals in the age groups indicated above is very high. All three studies failed to reveal the potential difficulty if the method is used during the period when the young are ten to twelve months of age and their bacula are enlarging. Thus, during the late December to March period, the peak of the trapping season in some areas, ageing mink by means of bacular conformation should be done with care. The proportion of young of the year detected by baculum conformation at this time would probably be an under-estimate of that in the population. Other criteria (such as development of supra-sesamoid tubercles on the femur, closure or lack of closure of the jugal-squamosal suture, etc., Greer, 1957) should be used. Since age ratios of young-of-year to adult individuals are important data in population analyses and in game management considerations, accuracy in their determination is essential.

I wish to thank W. L. Trent for providing a number of mink skeletons for study. G. E. Woolfenden, E. A. Munyer and P. W. Parmalee offered several suggestions in preparation of the manuscript, for which I am most grateful. All specimens examined in this report are presently in the collection of the Zoology Department, North Carolina State University, Raleigh.

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Manuscript received Nov. 24, 1967.

CHARACTERISTICS OF LEECH BLOOD PROTEIN

(*Haemopis Marmoratis, SAY*)

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ABSTRACT. — The 80% ethyl alcohol extraction of the blood of the leech, *Haemopis marmoratis* (Say) yielded but one free amino acid, aspartic acid, when subjected to two dimensional chromatography.

The protein fraction when electrophoresed with 300 V for 45 minutes indicated the presence of three different proteins. The hydrolysate of the protein fraction, after acid hydrolysis and two dimensional chromatography, yielded fourteen different amino acids.

The amino acids present were alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, proline, serine, threonine, tyrosine, valine and an unknown amino acid. The consistency of the chromatographs indicated the presence of the same proteins as substantiated by the electrophoretic study, in all fourteen leeches tested.

Haemopis marmoratis is an amphibious leech which leaves the water part of the time to live in damp soil or under stones, feeding on worms which they swallow whole (Mann, 1962). It is probably possible for this species to take blood, but they are generally thought to favor a more carnivorous life (Moore, 1901 and Mann, 1962).

Very little is known of the composition and qualities of blood in leeches. The role of hemoglobin in oxygen transport can be assumed from similarities in function in other organisms. On the other hand the buffering capacity, the isoelectric point and pH levels of the blood, all related to the amino acid content of the blood protein, are unknown. The blood of the leech, *Haemopis marmoratis* (Say) was analyzed by use of chromatographic and electrophoretic techniques in order to determine the free amino acid and protein characteristics of blood.

METHODS AND MATERIALS

All specimens of *H. marmoratis* were collected from the banks of the Kishwaukee River north of DeKalb in late spring and maintained in small tanks of running "tap" water kept at approximately 18°C. No provisions were made to feed the leeches after collection.

Each leech was narcotized with 15% ethyl alcohol for 15 minutes before being bled. Blood was diluted with 0.8% saline solution and stirred vigorously with a glass rod prior to analysis.

Blood samples of one to two ml. were extracted with portions of boiling 80% ethyl alcohol, the extraction being carried out for 1 hour with the aid of a shaker (Hughes, Lynch and Somers, 1958). After evaporation the concentrate was diluted to 3 ml. with 50% isopropyl alcohol. The extract containing the free amino acids was spotted on Whatman No. 1 filter paper sheets. Two dimensional chromatograms, based on the methods of Levy and Chung (1953), were utilized for the amino acid analyses.

Acid hydrolysis of the protein

The protein portion of the blood was refluxed in 10 ml. of 6N HCL on a steam bath for twelve hours (Crossan and Lynch, 1958, Lynch and Bogitsh, 1962, and Hughes *et al.*, 1958). The hydrolysate was evaporated to dryness and the residue dissolved in 1 ml. of 10% isopropyl alcohol. The mixture was spotted on filter paper and developed according to the procedure of Levy and Chung (1953). The amino acids in the free amino acid pool and the protein hydrolysates were compared against chromatograms prepared from known amino acid standards developed by the same procedures.

Electrophoresis of the protein portion

The precipitated portion of the extraction containing the protein fractions was suspended in 0.8% saline solution and vigorously stirred with a glass rod. The electrophoretic techniques described by Block, Durrum and Zweig, (1958) were used to determine the number of

proteins present in the blood. Gelman's rapid electrophoresis chamber No. 51101 and Gelman's high resolution buffer No. 51104 were used according to the manufacturer's directions. Cellulose polyacetate electrophoresis support membrane was used in preference to filter paper because the cellulose acetate is demonstrably more rapid, discriminative and versatile than other available methods (Grunbaum, Kirk and Atchley, 1961).

pH determinations

The pH of the leech blood was determined with a miniature combination electrode introduced into the coelomic cavity of the leech and read on a Corning model 7 pH meter.

Results and Discussion

Eight chromatograms were prepared by building up thirty spots of the extracted fluid on each chromatogram. The resulting chromatograms indicated the presence of a single free amino acid, aspartic acid. The electrophoresed protein fraction indicated three proteins present. Since hemoglobin is present in leech blood, it would be fair to assume that the darkest band represented this portion of the blood. The two remaining bands appeared very light, indicating slight concentrations. They would seem to be of smaller molecular structure or of greater negativity or both to be able to migrate such a great distance from the first band.

The hydrolysate of the protein fraction established a chromatographic pattern of consistency throughout. The presence of the same 14 amino acids in eight of the 14 chromatograms (Table 1) substantiated the presence of the same three proteins as indicated by the electrophoretic strips. When lighter applications of hydrolysate were used proline and tyrosine were not detected. Apparently these amino acids are not found in great abundance as part of the

Table 1.—Amino Acids in the Protein Hydrolysate of Leech Blood.

	Leech													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Concentration of Hydrolysate*	L	L	H	H	H	L	H	L	H	H	H	H	L	L
Alanine**	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Asparagine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aspartic Acid	+	+	+	+	+	+	+	+	+	+	+	+	—	+
Cysteine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glutamic Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Iso-leucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leucine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	+	+	+	+	+	+	+	+	+	—	—
Methionine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Phenylalanine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Proline	+	+	+	+	+	—	+	—	+	+	+	+	—	—
Serine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Threonine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tryptophan	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyrosine	—	—	+	+	+	—	+	+	+	+	+	+	—	—
Valine	+	+	+	+	+	+	+	+	+	+	+	+	—	+
Unknown	—	+	+	+	+	+	+	+	+	+	+	+	+	+

*L = light concentration of hydrolysate; H = heavy concentration of hydrolysate.
** + = amino acid is present; — = amino acid is not present; 0 = amino acid did not appear but it may be present.

structure of the three proteins found in the blood. In thirteen of the fourteen chromatograms an unknown amino acid was detected but not identified.

An inspection of the amino acid chromatograms indicated the presence of two dicarboxylic acids; aspartic acid and glutamic acid. Also present was one dibasic amino acid, lysine. Cysteine, cystine and methionine were not present which indicated the absence of sulphur-bearing proteins. The dicarboxylic amino acids could constitute a major part of the two proteins which migrated the greater distance on the electrophoretic strips. Values for the pH of blood ranged from 6.70 to 7.35 with an average of 7.02.

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Manuscript received Dec. 9, 1967.

NEW PLANT RECORDS FROM SOUTHERN ILLINOIS

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ABSTRACT. — *Panicum scoparium*, *Isotria verticillata*, *Duchesnea indica*, *Dioclea multiflora*, *Bartonia paniculata*, and *Solidago sphacelata* are reported from Illinois for the first time. Numerous records for other rare species are included.

Extensive collecting in the southeastern tip of Illinois during the summer of 1967 resulted in the discovery of six species new to the Illinois flora. Many other plants were collected which represent significant extensions of range or additional records for rarely collected species. All but one of the records reported are from Pope or Massac Counties.

The primary collecting area in Pope County was the sandy Cretaceous Hills section in the southern part of the county. These hills rise about 150 feet above the lowlands to the south and are composed of Cretaceous and Tertiary sands and gravels. The dry sandy uplands have a flora unlike that found elsewhere in southern Illinois. Sandy seep springs are common in the valleys and form a rich "marshy" habitat. These two habitats account for a majority of the records included in this paper. Some additional records from Massac County were discovered in the small ravines along the Ohio River between Metropolis and Joppa.

All specimens cited have been deposited in the herbarium of Southern Illinois University (SIU).

SPECIES NEW TO ILLINOIS

Panicum scoparium Lam. This large conspicuous grass grows in old fields, and along roadsides, in the Cretaceous Hills section. It is found in low swales, along streams, and roadside ditches, where it is associated with *Rhexia mariana* and *Coreopsis pubescens*. *Panicum scoparium* was to be expected in southern Illinois since it was known to occur in adjacent Kentucky and Missouri. Collection data: 3½ miles southwest of Bay City, Pope County, Illinois, June 24, 1967, *Schwegman 1260*.

Isotria verticillata (Willd.) Raf. Although Illinois is within the general range of this rare and local orchid, it has not been previously collected in the state. It forms colonies in damp sandy soil in woods of the Cretaceous Hills section. Approximately 50 sterile plants were observed. Nearby springs contain sphagnum moss, *Thelypteris noveboracensis*, and *Solidago patula*. Collection data: 3¾ miles southwest of Bay City, Pope County, Illinois, June 24, 1967, *Schwegman 1262*.

Duchesnea indica (Andr.) Focke. This adventive species occurs as a spontaneous weed in a lawn at 21st Street and North Avenue in Metropolis. Collection data: Metropolis, Massac County, Illinois, April 24, 1967, *Schwegman 1132*.

Dioclea multiflora (T. & G.) C. Mohr. This large twining legume of the southeast is apparently local in occurrence. Gleason (1963) gives its range as "western Kentucky to Georgia and Louisiana." It was collected at the edge of a woods at the base of a small hill adjacent the Ohio River flood plain. It grows here with other vines such as *Cocculus carolinus* and *Gonolobus gonocarpos*. Collection data: 3 miles west of Metropolis, Massac County, Illinois, June 17, 1967, *Schwegman 1249*.

Bartonia paniculata (Michx.) Muhl. is a species of damp soil on the coastal plain. Steyermark (1963) lists this species from sandy spring-fed streams in Stoddard County, southeastern Missouri. The collection reported here is some 80 miles northeast of the Missouri stations and is the northernmost record in the Mississippi embayment. Another coastal plain species growing with it is *Triadenum tubulosum*. It was discovered in a marshy spring and for some distance along the small stream which emanated from it. Collection data: 4½ miles south of Bay City, Pope County, Illinois, August 27, 1967, *Schwegman 1388*.

Solidago sphacelata Raf. Although Fernald (1950) attributes this goldenrod to southern Illinois, Gleason (1963) gives a more eastern distribution, and Jones (1963) does not list it for Illinois. The author has observed it to be abundant in adjacent western Kentucky. It

was discovered at the base of a sandstone bluff at Dam 51 on the Ohio River at Golconda. Collection data: Golconda, Pope County, Illinois, August 27, 1967, *Schwegman 1390.*

Species New To Southern Illinois

Scleria triglomerata Michx. was formerly known only from the northern half of the state. It was found growing with *Polygala incarnata* in a dry sandy old field. Collection data: 3½ miles southwest of Bay City, Pope County, Illinois, June 24, 1967, *Schwegman 1259.*

Physocarpus opulifolius (L.) Maxim. This attractive shrub occurs locally in northern Illinois with the southernmost previous station being in Wabash County. It is abundant along Copperous Branch of Lusk Creek southeast of Eddyville. Collection data: Copperous Branch, southeast of Eddyville, Pope County, Illinois, August 16, 1967, *Schwegman 1362.*

Oxalis grandis Small. This is the second collection of this plant from Illinois; it was previously collected in Wabash County by Schneck. It grows in a deep sandstone ravine along Little Lusk Creek known locally as Martha's Woods. It is abundant at this station, but only a single plant was found in flower. Collection data: Martha's Woods, 4 miles ENE of Eddyville, Pope County, Illinois, April 23, 1967, *Schwegman 1029.*

Rarely Collected Species

Panicum malacophyllum Nash. Mohlenbrock and Ozment (1967) reported this grass from Illinois for the first time. It grows in dry sandy soil. Collection data: 3½ miles southwest of Bay City, Pope County, Illinois, June 24, 1967, *Schwegman 1258.*

Elaeocharis wolfii (A. Gray) Patterson. Mohlenbrock and Draplik (1962) list this sedge from five widely scattered counties in the state. It is rather abundant in a wet ditch near Mermet. Collection data: Mermet Wildlife Refuge, Massac County, Illinois, May 22, 1967, *Schwegman 1194.*

Cimicifuga rubifolia Kearney. Formerly only found in the state in deep ravines of Pope County, this rare species was collected at Rock Spring in the northwest corner of Massac County. It grows in rich woods on a north-facing limestone hillside. Collection data: 1½ miles north of Boaz, Massac County, Illinois, August 29, 1967, *Schwegman 1397.*

Prunus virginiana L. On the basis of a sterile specimen, Ozment (1967) reports this shrub from Hardin County, its only other southern Illinois station. I collected it on a north-facing sandstone ledge along Hayes Creek. Collection data: 1½ miles southwest of Eddyville, Pope County, Illinois, May 22, 1967, *Schwegman 1149.*

Euonymus americanus L. This shrub grows abundantly along the edge of a cypress swamp. Collection data: 2¼ miles north of Belknap, Johnson County, Illinois, August 18, 1967, *Schwegman 1376.*

Buchnera americana L. Bluehearts was found growing in dry sandy fields with *Scleria pauciflora.* This is the first record from southeastern Illinois. Collection data: 3½ miles southwest of Bay City, Pope County, Illinois, June 24, 1967, *Schwegman 1256.*

Melanthera hastata Michx. Previously known only from low woods along the Ohio River in Pulaski County, this southern composite was collected in a similar habitat in Massac County. It appears to be native and grows with other southern plants such as *Halesia carolina.* Collection data: Thornton's Ravine, 5 miles west of Metropolis. Massac County, Illinois, August 6, 1967, *Schwegman 1349.*

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Manuscript received Sept. 30, 1967.

NEW AND UNUSUAL *CAREX* RECORDS FOR ILLINOIS

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ABSTRACT. — Six taxa of the genus *Carex* are reported for the first time from Illinois as a result of a study of undetermined specimens at the Missouri Botanical Garden.

While annotating specimens of *Carex* for the Illustrated Flora of Illinois, an interesting group of undetermined Illinois specimens was found at the Missouri Botanical Garden. This paper reports the more interesting finds.

Fifty-one specimens were in the undetermined group. Of these, six were of taxa unreported from Illinois and fourteen others were county records.

Taxa New to Illinois

Caxex flava L. var. *fertilis* Peck. This taxon of northeastern North America has been reported from nine counties of Indiana by Deam (1940). He indicates it is "on marly lake borders and in marshes rich in marl." A specimen collected in sand in Lake County, Illinois, by M. C. Jensen in May, 1895, is the only one known from Illinois. In the rank of species, this taxon is known as *C. cryptolepis* Mack. Variety *fertilis* is not always readily distinguished from var. *flava*. It generally can be recognized by its nearly hidden pistillate scales and smaller pistillate spike (less than 1 cm thick). Furthermore, the perigynia average slightly shorter in var. *fertilis*, and the perigynial beak is essentially entire. All spikes in the Illinois material have a tuft of staminate flowers at the apex. This taxon belongs to Section Extensae, a group characterized by three stigmas, sharply trigonous achenes, a prolonged tubular-sheathing bract, and reflexed perigynia. In addition to *C. flava* var. *fertilis*, Section Extensae is represented in Illinois by *C. viridula*. The latter is readily distinguished by its proportionately shorter perigynial beak.

Carex garberi Fern. This is another sand-inhabiting species of northeastern North America which was known previously from northwestern Indiana where Deam (1940) says it is "infrequent in the lake area (mostly in the dune region) where it grows along the wet sandy edges of swales in the dunes and on old lake beds". This is the plant that has been erroneously called *C. hassei*. The distinctive character of *C. garberi* is its white-papillate, beakless perigynium. This species belongs to Section Bicolores. This is one of the few sections of subgenus *Carex* which has 2 stigmas and a lenticular schene. *Carex aurea* Nutt., a species known from two collections in Lake County, also belongs to this section. It differs from *C. garberi* by its staminate terminal spike and its golden-orange perigynia. The Illinois specimen reported here of *C. garberi* was collected in Cook County at Lakeview on June 20, 1887, by L. H. Pammel.

Carex pennsylvanica Lam. var. *distans* Peck. This taxon differs from var. *pennsylvanica* by its significantly longer perigynia (over 3 mm) and the perigynial beak nearly as long as the body. As a species this taxon is known as *C. lucorum* Willd. The collection reported here from Illinois was made in Lake County at Lake Forest in 1897 by *M. C. Jensen 99*. This taxon is a variety known from the northeastern states. It has been found in Wisconsin.

Carex setacea Dewey. This poorly known species has the general appearance of *C. vulpinoidea* but differs markedly in its extremely slender perigynia. Since achenes at best are very poorly developed it has been suggested that *C. setacea* is a sterile hybrid between *C. vulpinoidea* and *C. annectens*. The specimen reported here was collected in Lake County at Lake Forest in 1896 by *M. C. Jensen 102*.

Carex buxbaumii Wahlenb. f. *heterostachya* Anderss. This variant differs from typical *C. buxbaumii* by having the terminal spike staminate throughout. The first record in Illinois is based upon a collection from Hancock Coun-

ty at Augusta by S. B. Mead in 1842.

Carex cristatella Britt. f. *catelliformis* (Farw.) Fern. This taxon is distinguished from typical *C. cristatella* by the spikes which are arranged in a moniliform head. The collection reported here was made by H. Eggert on May 8, 1892, from French Lake in St. Clair County.

Additional Illinois county records from the undetermined Carices at the Missouri Botanical Garden follow.

Carex aggregata Mack. Madison.

Carex albolutescens Schwein. Macon, St. Clair.

Carex amphibola Steud. var. *rigida* (Bailey) Fern. St. Clair.

Carex brachyglossa Mack. St. Clair.

Carex convoluta Mack. Stephenson.

Carex crinita Lam. LaSalle.

Carex emoryi Dewey. Hancock.

Carex granularis Muhl. St. Clair.

Carex lacustris Willd. Madison.

Carex molesta Mack. St. Clair.

Carex projecta Mack. Stephenson.

Carex tribuloides Wahlenb. LaSalle, Randolph.

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Manuscript received Sept. 15, 1967.

NOTES ON THE FLORA OF EXTREME SOUTHERN ILLINOIS

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ABSTRACT. — Four species of vascular plants are reported new for Illinois. These are *Gymnopogon ambiguus*, *Panicum mattamuskeetense*, *Cerastium tetrandrum*, and *Waldsteinia fragarioides*. Other species new to southern Illinois, and some species seldom collected in southern Illinois, are included.

Botanizing in the southernmost counties of Illinois during the summer of 1966 and spring of 1967 turned up four species of vascular plants new to the Illinois flora. Several species new to southern Illinois, and many additional records for plants seldom collected in this area, are also reported in this paper. Brief descriptions are given for some of the collecting sites. All specimens are in the herbarium of Southern Illinois University (SIU).

Burke Branch is a tributary of Barren Creek about three miles southwest of Bay City in southern Pope County. Habitats along this branch range from open sunny prairie situations, to deep wooded ravines. In the latter habitat black cohosh (*Cimicifuga rubifolia*) and *Solidago rugosa* occur. One prairie-like community, in a sandy overflow area along the creek, contains big bluestem (*Andropogon gerardii*), *Gaura biennis*, dwarf willow (*Salix humilis*), and yellow gentian (*Gentiana flavida*) among others. Beak rush (*Rhynchospora capitellata*), St. John's-wort (*Hypericum lobocarpum*), and *Coreopsis pubescens* grow in the creek bed, and along its banks.

Hayes Creek forms a small gorge southwest of Eddyville in Pope County. Sandstone ledges rise directly from the creek to heights of forty feet or more in several places. Some of the ledges face north, and support vegetation typical hundreds of miles to the north. Beds of sphagnum moss grow on the wider ledges, while narrow ones support ground pine (*Lycopodium porophilum*), cinnamon fern (*Osmunda cinnamomea*), hay-scented fern (*Dennstaedtia punctilobula*), and closed gentian (*Gentiana andrewsii*). *Selaginella apoda* is particularly abundant. It grows on moist vertical faces of sandstone.

Many of the other collecting areas cited have been described in a previous paper (Schwegman & Mohlenbrock, 1966).

Species New to Illinois

Gymnopogon ambiguus (Michx.) BSP. Beard grass forms a conspicuous grass community with *Aristida purpurascens* along a small creek. Although it is known from as far north as southern Ohio, Kentucky, and southern Missouri (Gleason 1963), it has not previously been found in Illinois. Collection data: Pope County, along Burke Branch, November 16, 1966, *Schwegman 1088*.

Panicum mattamuskeetense Ashe. This grass was found growing along a levee with *Wisteria macrostachya*. Hitchcock (1950) gives its distribution as the coastal plain from Massachusetts to South Carolina, and in southern Indiana. Collection data: Massac County, Mermet Wildlife Refuge, May 17, 1966, *Schwegman 365*.

Cerastium tetrandrum Curtis. This adventive from Europe is apparently spreading across the eastern United States. In 1950, Fernald reported it only from southeastern Virginia. Then in 1957, Steyermark discovered it in Missouri. On April 7, 1967, Mohlenbrock found it along a highway in Union County, Illinois. This species very closely resembles *C. viscosum*, but careful study of the flowers reveals the presence of only five stamens (in the Illinois collection) instead of the ten typical for *C. viscosum*. Collection Data: Union County, along west side of Illinois Route

127, ¾ mile south of Alto Pass, *Mohlenbrock 13222.*

Waldsteinia fragarioides (Michx.) Tratt. The barren strawberry has a limited distribution in the midwest. Fernald (1950) gives its range in this area as Ontario to Minnesota, south locally to Indiana and Missouri. Steyermark (1963) describes the Missouri plants as very rare relict colonies surviving only in a few choice situations. The plants reported here are on a north-facing ledge about 20 feet above Hayes Creek. They form a dense colony 7 feet long and 1 foot wide. Adjacent ledges support *Lycopodium porophilum*, *Mitchella repens*, and *Sedum telephioides*. Collection Data: Pope County, along Hayes Creek, southwest of Eddyville, October 1, 1966, *Schwegman 1961.*

Species New to Southern Illinois

Zannichellia palustris L. The horned pondweed is an infrequent species in northern Illinois, but the collection reported here is the first from southern Illinois. Collection data: Lake Murphysboro State Park, *D. Tindall s.n.*

Carex conoidea Schk. This sedge is northern in distribution, and was previously unknown from the southern half of Illinois. Collection data: Massac County, shallow temporary pool on post oak flats, Mermet Wildlife Refuge, April 28, 1966, *Schwegman 252.*

Habenaria lacera (Michx.) Lodd. This orchid was found around "marshy" springs, and in moist soil along spring branches. Winterringer and Evers (1960) show no record of it south of Vermilion County. Collection data: Pope County, near Massac Lookout Tower, June 19, 1966, *Martha and John Schwegman 498;* Massac County, ¼ mile southeast of Mill Spring, June 25, 1966, *Schwegman 526.*

Habenaria flava (L.) R. Br. There seems to be no record of this species south of Wabash County. It grows in a colony of about 50 plants, of which only one flowered in 1966. It is associated with *Onoclea sensibilis* in a rich alluvial forest. Collection data: Massac County, Black Bottom, 2½ miles southeast of Unionville, July 30, 1966, *Schwegman 728.*

Lespedeza simulata Mack. & Bush. Jones (1963) reports this plant only from Crawford County. In southern Illinois it grows in a hill prairie with *Acerates viridiflora, Lespedeza virginica*, and *L. capitata.* Gleason (1963) suggests that it may be a hybrid of the latter two species. Collection data: Pope County, Millstone Knob, September 11, 1966, *Schwegman 989.*

Species Seldom Collected in Southern Illinois

Paspalum lentiferum Lam. Mohlenbrock, *et al.* (1962) reported this grass from Pulaski County for its first Illinois station. It was considered adventive at this site. In Massac County it grows in wet openings on post oak flats as well as wet roadside ditches. It appears to be native in the former habitat. Collection data: Massac County, Mermet Wildlife Refuge, August 13, 1966, *Schwegman 799.*

Carex caroliniana Schw. This sedge was formerly known in Illinois only from Jackson County. Collection data: Massac County, Mermet Wildlife Refuge, May 17, 1966 *Schwegman 361.*

Carex laevivaginata (Kükenth.) Mack. Collection data: Pope County, near Massac Lookout Tower, in wet soil near a spring, July 9, 1966, *Schwegman 608.*

Allium tricoccum Ait. This more northern onion had its only southern Illinois station in a deep ravine in Jackson County. The collection reported here is in a woods on a gentle southwest-facing slope beneath a limestone bluff. *Asarum reflexum* and *Hybanthus concolor* are associated with it. Collection data: Massac County, Dan Mathis farm, 4 miles north of Mermet, May 12, 1966, *Schwegman 323* (leaves), and June 28, 1966, *Schwegman 546* (flower and fruit).

Tipularia discolor (Pursh.) Nutt. Collection data: Massac County, along a small stream in woods, NW¼, Sect. 22, T 15 S, R 6 E, March, 1966, *Schwegman 179.*

Triphora trianthophora (Sw.) Rydb. Collection data: Johnson County, rich woods, along the south side of Black Slough northwest of Belknap, September 25, 1966, *Schwegman 1031.*

Hibiscus moscheutos L. Jones (1963) reports this from Williamson County for its only other southern Illinois station. It is the commonest species of *Hibiscus* around Mermet Lake. Collection data: Massac County, Mermet Wildlife Refuge, July 27, 1966, *Schwegman 708.*

Hypericum lobocarpum Gatt. This was formerly known in Illinois only from Massac County (Mohlenbrock and

Voigt, 1959). Collection data: Pope County, along Burke Branch, September 5, 1966, *Schwegman 948.*

Steironema radicans (Hook.) Gray. Collection data: Johnson County, Goose Pond, 4 miles southwest of Vienna, in low swampy woods, August 21, 1966, *Schwegman 851.*

Gentiana flavida Gray. This is the second collection of this gentian from southern Illinois. Collection data: Pope County, along Burke Branch, September 5, 1966, *Schwegman 946.*

Salvia pitcheri Torr. Collection data: Massac County, on dry limestone ledges, Dan Mathis farm 4 miles north of Mermet, September 1, 1966, *Schwegman 915.*

Solidago rugosa Mill. This rare goldenrod was growing along a creek in a wooded ravine. Collection data: Pope County, along Burke Branch, September 24, 1966, *Schwegman 1032.*

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Manuscript received Sept. 29, 1967.

ADDITIONAL RECORDS OF THE FERNS OF CUMBERLAND COUNTY

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ABSTRACT. — Field work in the Embarras River Valley in Cumberland County, Illinois has revealed five ferns and seven forms of ferns which have not been previously reported for the county. A form of the silvery spleenwort showing a forked pinna has not been previously reported.

Mohlenbrock (1967) lists only seven ferns as occurring in Cumberland County. Wunderle (1967) reported an additional six ferns as occurring in the county. The purpose of the paper is to present information on new ferns found during 1967, their forms, and the abundance of the ferns found in the Embarras River Valley. The fern habitats of all species that have not been previously reported are included because of the rapid depletion of bottomland timbers to make way for the new Lincoln Reservoir which will flood the area. The species preceded by an asterisk (*) are those which have not been previously reported as occurring in Cumberland County. The specimens are in the herbarium of the author. The order of the fern families and nomenclature are based largely on that of Mohlenbrock (1967) and Wherry (1961).

OPHIOGLOSSACEAE

Botrychium dissectum Spreng. var. *obliquum* (Muhl.) Clute. The grape fern is rather rare in occurrence in the Embarras River Valley, but when it is found it occurs with *Botrychium virginianum* in moist bottomland. Wunderle 67-11.

Botrychium virginianum (L.) SW. The rattlesnake fern is one of the most common ferns growing in the valley. It can be found growing in moist bottomland and north-facing upland slopes. Wunderle 67-3.

OSMUNDACEAE

**Osmunda claytoniana* L. The interrupted fern is occasionally found in moist, north-facing low woodlands at Gold Hollow and on a moist, west-facing slope at Copper Cave. This fern totals only ten plants in the two areas. Wunderle 67-4.

POLYPODIACEAE

Adiantum pedatum (Tourn.) L. The maidenhair fern is a very common fern of moist lowlands and ravines. This fern in Cumberland County is generally an indicator of other moisture loving ferns. Wunderle 67-5.

Polystichum acrostichoides (Michx.) Schott. The Christmas fern is the second most abundant fern in the county. The fern grows on northern slopes and on south facing slopes if the ravine is deep enough to produce enough shade and moisture. Wunderle 67-6. Besides the common form, there are four variants that have been found and are thought to be caused by the environment.

*forma *crispum* Clute. A frond of this form which has the pinnae wavy was found at Gold Hollow growing from a typically normal plant. Wunderle 67-7.

*forma *incisum* (Gray) Gilbert. The frond of this form are more coriaceous and have coarsely serrate pinnae. A frond of this variant was found growing from a typical plant at Gold Hollow. Wunderle 67-8.

*forma *multifidum* Clute. The pinnae of this form are deeply pinnatifid. Three fronds of this variant were growing from a plant with two typical fronds in a moist ravine in the SW, SE, NW, 27, Pleasant Grove Township. Wunderle 67-9.

*forma *cristatum* Clute. This variant has the pinnae forked and the tip of the frond more or less forking. It appears that the forking of the frond may have resulted from injury. This form was growing in a moist ravine in the SW, SE, NW, 27, Pleasant Grove Township. This form has also

been reported from southern Illinois by Mohlenbrock and Wunderle (1967) Wunderle 67-10.

Onoclea sensibilis L. The sensitive fern has been found growing in only one area of Gold Hollow and that represented by an extensive colony in a north-facing bottomland timber. Wunderle 67-20.

**Thelypteris hexagonoptera* (Michx.) Weath. The broad beech fern is rarely found but when located it is usually located in a moist ravine with heavy shade. There are three colonies of this fern in Gold Hollow. Wunderle 67-12.

*forma *Simonii* Reed. This form has the pinnatifid pinna forking. This variant was growing with typical specimens. This form has not been previously reported in Illinois. Wunderle 67-13.

**Athyrium pycnocarpon* (Spreng.) Tidestr. The narrow-leaved spleenwort is extremely rare in the county and has been found in moist, north-facing bottomland. There are only eight plants of this fern found at Gold Hollow. It can be found growing with the silvery spleenwort which is abundant at this site. Wunderle 67-14.

Athyrium thelpteriodes (Michx.) Desv. The silvery spleenwort is a frequently found fern in moist, wooded ravines. One ravine in the SW, SE, NW, 27, Pleasant Grove Township has specimens which have fronds in excess of 40 inches. Wunderle 67-15.

*A rare variant has a forked pinna midway on the rachis. The forked pinna has one shank parallel to the main axis and the other shank forming a 45 degree angle with the main axis. This variant has not been previously described and therefore is new for Illinois. Wunderle 67-17.

**Athyrium filix-femina* (L.) Roth. var. *rubellum* Gilb. The lady fern is rather rare in the county and has been growing in a west-facing, moist, bottomland of Copper Cave. Wunderle 67-16.

Asplenium platyneuron (L.) Oakes. The ebony spleenwort is not common but can be found in dry woodlands or shaded, exposed sandstone crevices. Wunderle 67-18.

**Woodsia obtusa* (Spreng.) Torr. The common woodsia is occasionally found on shaded, exposed sandstone and dry woods. Wunderle 67-19.

Cystopteris fragilis (L.) Bernh. var. *protrusa* Weath. The fragile fern is the most common fern in Cumberland County. It grows in moist woodlands and dry, shaded slopes. Wunderle 67-1.

*forma *cristata* (Lowe) Weath. This form shows the frond forked at the tip and is new for Illinois. Wunderle 67-2.

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Manuscript received Nov. 30, 1967.

THE ELECTROMAGNETIC FIELD OF A BUNCHED ELECTRON BEAM

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ABSTRACT. — From qualitative considerations of phenomona taking place in the electron beams of traveling wave amplifiers (TWAs), general expressions for the current and charge densities in such beams are deduced, in the form of superpositions of simple harmonic traveling waves. It is shown that the electromagnetic fields of such beams may be calculated from the classical retarded potentials, with an error which vanishes with increasing distance along the beam.

There is a class of microwave electron devices which leads one to interesting and useful problems in analysis. Among the most important of these is the traveling wave amplifier (TWA). Let us give a brief general description of such tubes. A TWA consists essentially of two ingredients, *viz.*, a wave guide structure along which electromagnetic waves can propagate, together with a stream of electrons flowing near to and along the whole length of the structure. The wave guide is designed in such a way that, among the class of waves which can propagate along it, there is at least one having a phase velocity small compared to the phase velocity of plane waves in vacuum. The electrons in the stream are given an average or drift velocity along the guide which is just equal to the phase velocity of the slow wave just mentioned. (A particle of the stream thus experiences a constant or at most a slowly varying phase of the field as it travels along the guide.) At the input end of the guide, matters are arranged so that a signal may be introduced, which then propagates along it. At the output end, the signal is extracted *via* a suitable matched load (so that there are only traveling waves, never standing waves, in the guide). It is found that when the beam and wave velocities are equal, or very nearly so, the amplitude of the extracted signal is large compared with the input, so that the device behaves as an amplifier. Indeed, it will amplify at all frequencies for which the mentioned phase and beam velocities are nearly equal, and its usefulness derives from the fact that it is possible to achieve such a correspondence in velocities over remarkably wide frequency intervals. For further technical details, we must refer to the works of Beck [1958], Slater [1950], Pierce [1950] and the literature cited there.

In general, analysis of phenomena in TWAs requires one to solve a non-linear system of hyperbolic partial differential equations. These are Maxwell's equations for the electro magnetic field vectors $\overline{E}$ and $\overline{H}$, supplemented by equations which relate $\overline{E}$ and $\overline{H}$ to the charge density δ and velocity $\overline{v}$ in the electron beam, together with suitable boundary conditions

at the edge of the beam and on the surface of the wave guide. Examples of such systems for particular TWAs may be found in the above cited works as well as in Chu and Jackson [1948]; Birdsall and Whinnery [1953].

The difficulty of obtaining even crude approximate solutions of the non-linear equations has led the writer to consider approximate *synthetic* methods of solution: instead of attempting to solve the non-linear system for the four unknown quantities $\overline{E}$, $\overline{H}$, δ, $\overline{v}$, we may instead consider the fields $\overline{E}$ and $\overline{H}$ which are associated with *given* beam densities and velocities δ and $\overline{v}$, wherein the latter contain certain parameters at least partially adjustable to given boundary conditions. The most important general phenomenon which takes place in the electron beam of a TWA (and, indeed, in certain other electron beam devices as well [3]) is *bunching* of the particles. Suppose — as is well-approximated in actual TWAs — that our beam of electrons is cylindrical in shape, occupying the domain $0 \leq r \leq B$, $z \geq 0$ in cylindrical coordinates r,ϕ,z (Fig. 1) (we may imagine $z = 0$ to be the location of the TWA input). Such a beam is said to be *bunched* if the particles are not distributed uniformly with distance along the z-axis, i.e., if the charge density $\delta = \delta(r,\phi,z,t)$, at any instant t, depends on z. (Such bunching occurs in TWAs because as the electrons travel along substantially in phase with the field, they tend to crowd into regions where the force on them is least—in the course of which, they give up energy to the field.)

In this paper, we study the fields $\overline{E}$ and $\overline{H}$ which are generated by cylindrical bunched beams. We first set forth some general relations from classical electromagnetic theory.

2. *Fields Of Given Charge And Current Distributions. Vector And Scalar Potentials.* We follow the well-established notation and terminology of Stratton [10], and use MKS units. (The dimensions of the physical quantities used are set forth explicitly.)

When the current density $\overline{J}$ (amperes/(meter)^2) and the charge density δ (coulombs/(meter)^3) are prescribed, the associated field vectors $\overline{E}$ (volts/meter) and $\overline{H}$ (ampere-turns/meter) are most simply obtained indirectly, *via* the vector and scalar potentials $\overline{A}$ (webers/meter) and Φ (volts). The latter satisfy the non-homogeneous wave equations

$$\nabla\,\nabla\cdot A - \nabla\times\nabla\times A - \mu\epsilon\frac{\partial^2\overline{A}}{\partial t^2} = -\mu\overline{J} \tag{1}$$

$$\nabla^2\Phi - \mu\epsilon\frac{\partial^2\Phi}{\partial t^2} = -\frac{\delta}{\epsilon} \tag{2}$$

on any domain of vanishing conductivity, which is supposed. (ϵ = permittivity of the medium, farads/meter; μ = permeability of the medium,

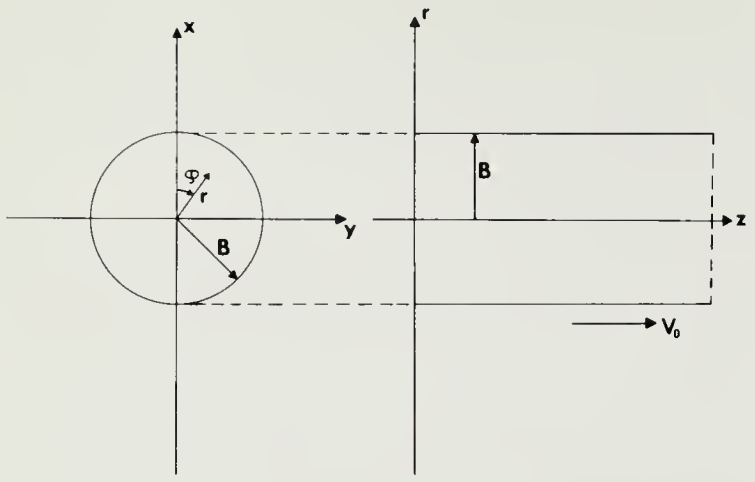
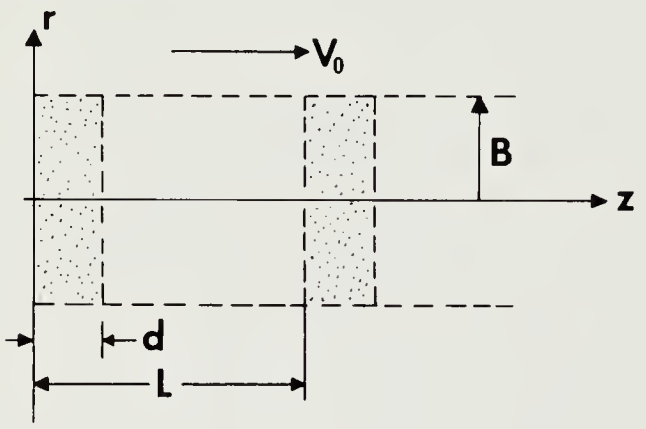


FIGURE 1. — Coordinates for cylindrical electron beams of radius r = B



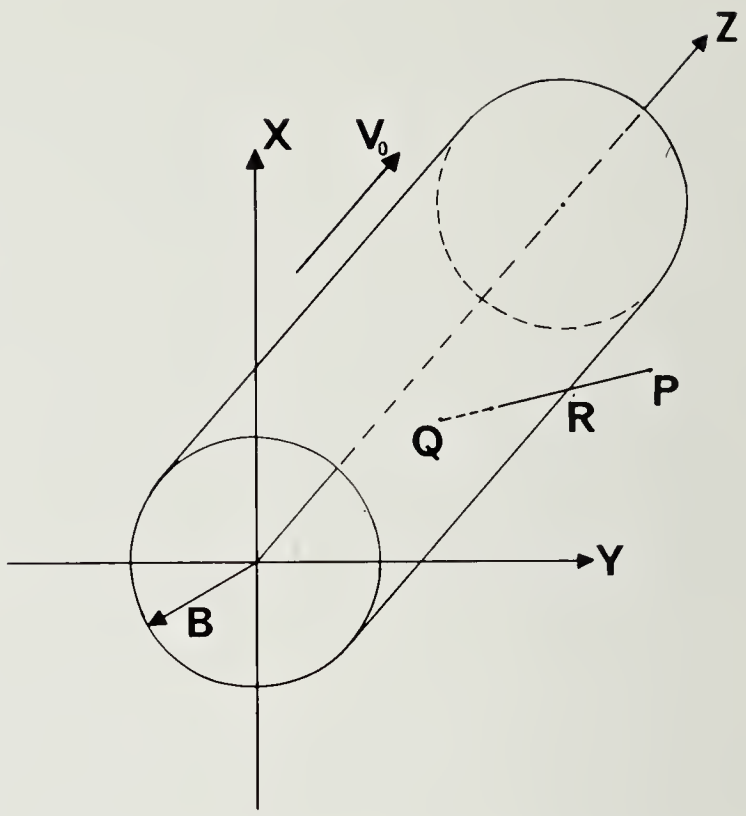


FIGURE 2. — Points P and Q, and distance R, in the retarded potentials (6), when D is the cylindrical domain of Fig. 1. Point Q runs over D while P represents an arbitrary but fixed point. (P may lie within as well as outside D.)

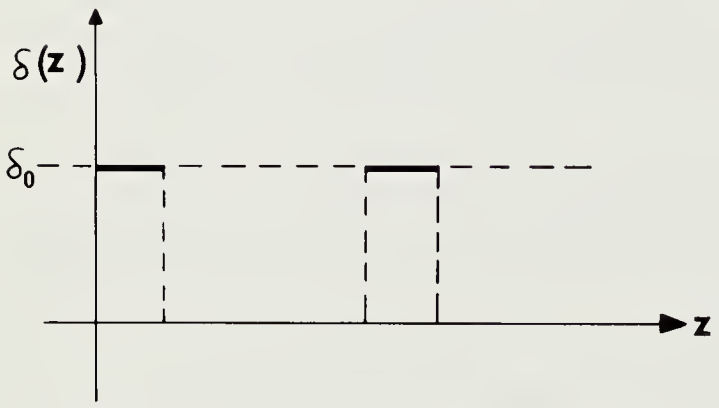


FIGURE 3. — A schematic diagram of events in a simple bunched beam. The charge is concentrated in periodically spaced discs of thickness d (Fig. 3A), with constant density in each disc (Fig. 3B)

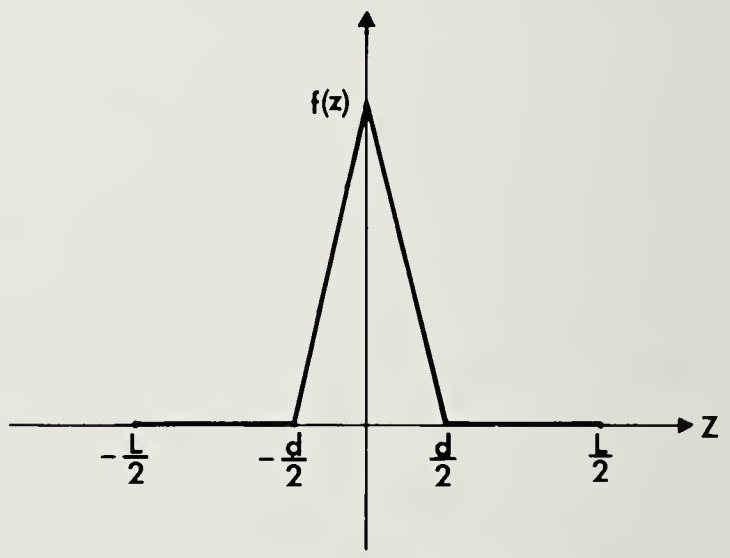


FIGURE 4. — Illustrating a simple periodic function f(z) used to construct a charge density function.

henrys/meter, both being positive constants.) The vector operator on the left-hand side of (1) reduces to the Laplacian operating on the separate components of $\overline{A}$ only in rectangular coordinates. However, A_z is a cylindrical as well as a rectangular component, so that

$$\frac{1}{r}\frac{\partial}{\partial r}\, r\, \frac{\partial A_z}{\partial r} + \frac{1}{r^2}\frac{\partial^2 A_z}{\partial \Phi^2} + \frac{\partial^2 A_z}{\partial z^2} - \mu\epsilon\frac{\partial^2 A_z}{\partial t^2} = -\mu J_z \qquad (3)$$

If $\overline{A}$ and Φ are solutions of (1) and (2), then it may be shown [10] that the vectors

$$\overline{E} = -\nabla\Phi - \frac{\partial^2\overline{A}}{\partial t}\,,\quad \mu\overline{H} = \nabla\times\overline{A} \tag{4}$$

are solutions of Maxwell's equations provided the densities $\overline{J}$ and δ satisfy the conservation law

$$\nabla\cdot\overline{J} + \frac{\partial\delta}{\partial t} = 0 \tag{5}$$

throughout their domain.

Well-known particular solutions of (1) and (2) are the *retarded potentials of* Kirchhoff and Lorentz ([5], [10]), described as follows. Let P be a fixed but arbitrary point, and Q any point of the domain D of $\overline{J}$ and δ. Then the potentials $\overline{A}$ and Φ at P, at any time t, are given by

$$\overline{A}(P,t) = \frac{\mu}{4\pi}\int_D \frac{\overline{J}(Q,t^*)}{R}\,d\tau\,,\quad \Phi(P,t) = \frac{1}{4\pi\epsilon}\int_D \frac{\delta(Q,t^*)}{R}\,d\tau \tag{6}$$

where the integrals are taken over the entire domain D of the distribution (see Fig. 2, for D a cylindrical domain). In (6), R = distance from P to Q, $t^* = t - \frac{R}{u}$, where $u = (\mu\epsilon)^{-\frac{1}{2}}$; u is the velocity of phase propagation of plane waves in an unbounded medium with permeability μ and permittivity ϵ.

It is clear from (6) that in general $\overline{A}$ has as many non-vanishing components as does the current density $\overline{J}$. However, if $\overline{J} = \overline{i}_z J$ (in cylindrical coordinates), then — since $\overline{i}_z$ is a constant vector — $\overline{A}$ itself also has only an axial component $\overline{A} = \overline{i}_z A$, where

$$A(P,t) = \frac{\mu}{4\pi}\int_D \frac{J(Q,t^*)}{R}\,d\tau \tag{7}$$

In the sequel, we shall consider a class of densities for which the integral (6) for Φ and (7) for the z-component A of $\overline{A}$ are both explicitly evaluable. In other cases, of course, one must work directly with (1) and (2), taking advantage of whatever special features any particular problem may possess.

We remark, finally, that the beams of TWAs exist only in high vacua. The nature of the medium is accounted for in our equations by the constants ϵ and μ which, in vacuum, have the values $\epsilon_0 = 8.854\cdot10^{-12}$ farads/meter, $\mu_0 = 1.257\cdot10^{-6}$ henrys/meter. However, the beam may be

surrounded by media having different permittivities, in which case the *average* value of ϵ (say, over a portion of a plane transverse to the beam) may differ from its value for vacuum. It will therefore suffice for the present to make no special assumption about them other than that they are positive constants.

3. *Representations For Densities In Bunched Beams.* We suppose now that we have a beam of electrons confined to the cylindrical domain $D : O \leq r \leq B,\ z \geq O$, as described above. Further, we suppose that the state of the beam is completely describable by a charge density δ and a one-dimensional current density $\overline{J} = \overline{i}_z J$, both of which are at least piece-wise differentiable functions of time t and position Q in D. If $\overline{v} = \overline{i}_z v$ is the velocity at any time and position in D, then $J = \delta v$. (It therefore suffices to specify any two of the three quantities J, δ and v.)

The simplest form of a bunched beam is found if we have a swarm of electrons, confined to D, all moving in the direction $z > O$ with constant velocity v_0, but in which the charge density at any particular time t depends on z. If $\delta = \delta(r,\varphi,z)$ at $t = O$ then, at any other time we have $\delta = \delta(r,\varphi,z - v_0 t)$, on account of the constant velocity v_0 at every point of the stream. Then the current density J at any point is simply δv_0. In turn, the simplest useful variation of δ for our purposes is that of a constant component on which a *periodic* function of $z - v_0 t$ is superposed. For example, the beam might consist of discs of charge of thickness d, at constant distance apart, with the whole configuration moving with constant velocity $v_0 > O$. Then, at $t = O$, the beam has the appearance sketched in Fig. 3, in which we have supposed the density to be constant ($= \delta_0$) in each disc. In this case,

$$\begin{aligned} \delta(z) &= \delta_0,\ O \leq z \leq d, \\ &= O\ ,\ d < z < L \end{aligned} \tag{8}$$

and if we define $\delta(z + L) = \delta(z)$, then $\delta(z)$ has a Fourier development

$$\delta(z) = \sum_{m=-\infty}^{\infty} A_m\, e^{i \frac{2m\pi}{L} z}$$

and then

$$\delta(z,t) = \sum_{-\infty}^{\infty} A_m\, e^{i \frac{2m\pi}{L}(z - v_0 t)} \tag{9}$$

where

$$A_m = \frac{1}{L} \int_O^L \delta(z) e^{-i(2m\pi/L)z}\, dz,\ m = O, \pm 1, \pm 2, \ldots$$

Let us notice an important restriction: if our expressions for δ are to represent *electron* densities, then we must have $-\delta \geq 0$, according to the usual sign convention for the charge of an electron. With this point in mind, we may assign to δ any other periodic distribution having a Fourier representation instead of the simple example (8).

Now expression (9) is a superposition of simple harmonic traveling waves of the form $\exp i(\beta_m z - \omega_m t)$, with $\beta_m = \frac{2m\pi}{L}$, $\omega_m = v_0\beta_m$, so that the component wave of index $m = 0$ has phase-shift β_m radians/meter and oscillates at the frequency $f_m = \omega_m/2\pi$ cycles/second. In particular, the fundamental frequency $\omega_1 = 2\pi v_0/L$, increasing directly with the beam velocity v_0 and inversely with the period L.

More generally, we need not assume that the density is strictly periodic in z, but may instead assign to each component of frequency ω_m its own particular phase β_m, and write

$$\delta = \sum_{-\infty}^{\infty} A_m e^{i(\beta_m z - \omega_m t)} \tag{10}$$

and, in fact, β_m is generally a function of the fundamental frequency ω_1. Furthermore, there is no reason why the amplitudes A_m need be constant across the beam and, in fact, they are in general functions of position r,ϕ transverse to the direction of propagation. Thus we write the more general expression, in place of (10),

$$\delta = \delta(r,\phi,z,t) = \sum A_m(r,\phi) e^{i(\beta_m z - \omega_m t)} \tag{11}$$

Further, in order eventually to build up suitable models of phenomena in TWAs, we need densities like (11) but in which the amplitude of each wave varies exponentially with z, say in the form

$$e^{\alpha_m(z)} e^{i(\beta_m z - \omega_m t)} \tag{12}$$

where the functions $\alpha_m(z)$ are continuous. Unfortunately, in (12) we have lost the representation of δ by simple harmonic traveling waves. If, however, we are only interested in events taking place over some finite length of the beam. we may suppose that $\alpha_m(z)$ (and hence $\exp \alpha_m(z)$) is periodic with a period D comparable to the length in question (i.e., with a period D large compared with the period L above, or compared with the largest

of the periods $2\pi/\beta_m$). Then if $\exp \alpha_m(z)$ has a Fourier development,

$$e^{\alpha_m(z)} = \sum_{k=-\infty}^{\infty} B_{mk}\, e^{i\frac{2\pi k}{D} z} \tag{13}$$

we substitute (13) into (11) and (12) and obtain a double series

$$\sum_{m,k} A_m(r,\phi) B_{mk}\, e^{i(\beta_{mk} z - \omega_m t)} \tag{14}$$

where $\beta_{mk} = \beta_m + \dfrac{2\pi k}{D}$. Our representation of δ by simple harmonic traveling waves is thus formally restored, while permitting us to assign quite general values to the factors $\alpha_m(z)$ over any given length D.

Finally, it may be the case that when the charge density — considered as a function of t — is resolved into its Fourier components, a *single* traveling wave at each frequency ω_m is inadequate to describe the distribution. Thus, at any harmonic $\omega_m = m\omega_1$ of the fundamental frequency ω_1, one may have several traveling waves. Then (14) is replaced by the more general expression

$$\sum_{m,k,p} A_{mkp}(r,\phi) e^{i(\beta_{mkp} z - \omega_m t)} = \sum_{m,k} A_{mk0} e^{i(\beta_{mk0} z - \omega_m t)} + A_{mk1} e^{i(\beta_{mk1} z - \omega_m t)} + A_{mk,-1} e^{i(\beta_{mk,-1} z - \omega_m t)} + \dots \tag{15}$$

It only remains to notice that since the beam occupies the whole cylinder $0 \le r \le B$, any physically realizable density must be periodic in ϕ with period 2π. Expanding the factor $A_{mkp}(r,\phi)$ in (15), we have a representation of the form

$$\delta(r,\phi,z,t) = \sum A_{mkpn}(r) e^{in\varphi}\, e^{i(\beta_{mkp} z - \omega_m t)} \tag{16}$$

where the sum is taken over all the indices m,k,p,n. If we assume a similar expression for the velocity v,

$$v(r,\varphi,z,t) = \sum B_{mkpn}(r) e^{in\varphi}\, e^{i(\beta_{mkpn} z - \omega_m t)} \tag{17}$$

then the current density $J = \delta v$ is formally of the same type with amplitude factors $C_{mkpn}(r)$ given by suitable sums of products of the A and B coefficients in (16) and (17).

Let us see how these representations correspond to what is known from existing theories of the TWA. These are all linearized, low-level theories (e.g., [4], [8]) from which the following general picture of events emerges: at a fixed frequency ω, the axial component E_z of the electric field, in the *absence* of the electron beam, is given by an expression of the type

$$E_z = \sum_{n,m} E_n(r)\, e^{in\varphi}\, e^{i(\beta_{nm} z - \omega t)} \tag{18}$$

Then when a cylindrical beam with only z-components of density and velocity is injected into the tube, each component wave of (18) breaks up into at least *four* waves, with the same number of waves in the representation for the charge density and velocity induced by the interaction between beam and wave. Moreover, the amplitude of each of these waves depends on z, either growing or decaying exponentially like $e^{\pm\alpha z}$ (α = const.), or remaining constant, as the whole field travels in the direction $z>0$. It will be seen that in our heuristic discussion leading up to (16) and (17), these are precisely the possibilities which have been accounted for and, in fact, are even of a more general class than the density and velocity expressions produced by linear analyses. (For example, we have allowed for the fact that the amplitude change with z may be of a much more general character than merely increasing or decreasing like $e^{\pm\alpha z}$.)

Now consider the space $\mathcal{F}$ of all pairs of well-defined expressions of the form

$$\begin{aligned} \delta(r,\varphi,z,t) &= \Sigma\, A(r) e^{in\varphi}\, e^{i(\beta z - \omega t)} \\ J(r,\varphi,z,t) &= \Sigma\, B(r) e^{in\varphi}\, e^{i(\beta z - \omega t)} \end{aligned} \tag{19}$$

with sums in (19) taken over suitable indices, and defined on $0\le r\le B$, and where each pair satisfies the conservation law

$$\frac{\partial J}{\partial z} + \frac{\partial \delta}{\partial t} = 0 \tag{20}$$

By a bunched beam, we shall understand any pair δ, J *of* $\mathcal{F}$.

4. *The Fields of Bunched Beams.* From (6) and (7), we have the integrals

$$A(P,t) = \frac{\mu}{4\pi}\int_D \frac{J(Q,t^*)}{R}\, d\tau\ ,\quad \Phi(P,t) = \frac{1}{4\pi\epsilon}\int_D \frac{\delta(Q,t^*)}{R}\, d\tau \tag{21}$$

for the potentials, from which the fields may be derived. Consider, for example, the scalar potential $\Phi(P,t)$. The density δ of (19) evaluated at

t* is

$$\delta(r,\varphi,z,t^*) = \Sigma\, A(r)\, e^{in\varphi}\, e^{i(\beta z-\omega t)}\, e^{ikR}$$

where $k = \omega/u$. If we substitute this expression in the integral (21), we have, upon interchanging orders of summation and integration

$$4\pi\epsilon\ \Phi\ (P,t) = \int_D \frac{e^{ikR}}{R}\, [A(r)\, e^{in\varphi}\, e^{i(\beta z-\omega t)}]\, d\tau \tag{22}$$

Expression (22) shows that the integrals (21) define linear operators on the densities δ and J. It suffices, therefore, to consider integrals of the form

$$I(P,t) = \int_D \frac{e^{ikR}}{R}\, \delta(Q,t)\, d\tau \tag{23}$$

where $\delta(Q,t)$ now represents an arbitrary one of the elementary components of the densities (19), and from which we then construct the potentials of "arbitrary" distributions in $\mathcal{F}$ by superposition.

To evaluate (23), let ρ,θ,ζ be cylindrical coordinates of Q in D : $0 \le r \le B$, $z \ge 0$, and r,φ,z coordinates of the arbitrary but fixed point P (which may or may not be in D). Then we have

$$\delta(Q,t) = A(\rho)\, e^{in\theta}\, e^{i(\beta\zeta - \omega t)}$$

where n is a fixed but arbitrary integer; since

$$R^2 = r^2 + \rho^2 - 2r\rho\cos(\theta - \varphi) + (\zeta - z)^2$$

(23) becomes

$$I = e^{-i\omega t} \int_0^B A(\rho)\rho\, d\rho \int_0^{2\pi} e^{in\theta}\, d\theta \int_0^\infty \frac{e^{ikR}}{R}\, e^{i\beta\zeta}\, d\zeta \tag{24}$$

First, consider the integral with respect to ζ in (24),

$$\int_0^\infty \frac{e^{ikR}}{R}\, e^{i\beta\zeta}\, d\zeta \tag{24a}$$

and make the change of variable $\zeta = x + z$, giving

$$\int_0^\infty \ldots = e^{i\beta z} \int_{-z}^\infty \frac{e^{ikR}}{R} e^{i\beta x}\, dx,\quad R^2 = r^2 + \rho^2 - 2r\rho\cos(\theta - \Phi) + x^2 \tag{25}$$

To evaluate (5), we use Sommerfeld's integral representation [6] for e^{ikR}/R, *viz.*,

$$\frac{e^{ikR}}{R} = \int_0^\infty J_0(\tau\sqrt{r^2+\rho^2-2r\rho\cos(\theta-\varphi)})\, \frac{e^{-|x|\sqrt{\tau^2-k^2}}}{\sqrt{\tau^2-k^2}}\, \tau\, d\tau \tag{26}$$

Upon substituting in (25) and interchanging orders of integration, (25) becomes

$$e^{i\beta z} \int_0^\infty J_0(\tau\sqrt{\ldots})\, \frac{\tau\, d\tau}{\sqrt{\tau^2-k^2}} \int_{-z}^\infty e^{i\beta x} e^{-|x|\sqrt{\tau^2-k^2}}\, dx \tag{27}$$

For the integral with respect to x, we write

$$\int_{-z}^{\infty} = \int_{-z}^{\infty} + \int_{-\infty}^{-z} - \int_{-\infty}^{-z} = \int_{-\infty}^{-\infty} - \int_{-\infty}^{-z}$$

so that

$$\int_{-z}^{\infty} e^{i\beta x} e^{-|x|\sqrt{\tau^2-k^2}}\, dx = \frac{2\sqrt{\tau^2-k^2}}{\tau^2+\beta^2-k^2} + e^{-i\beta z}\, \frac{e^{-z\sqrt{\tau^2-k^2}}}{i\beta+\sqrt{\tau^2-k^2}}$$

and we have for (25)

$$\int_0^\infty \frac{e^{ikR}}{R} e^{i\beta\zeta}\, d\zeta = e^{i\beta z} \int_0^\infty J_0(\tau\sqrt{\ldots}) \frac{2\tau\, d\tau}{\tau^2 + \beta^2 - k^2} \tag{28}$$

$$+ \int_0^\infty J_0(\tau\sqrt{\ldots}) \frac{e^{-z\sqrt{\tau^2-k^2}}\, \tau\, d\tau}{(i\beta + \sqrt{\tau^2-k^2})\sqrt{\tau^2 - k^2}} \tag{28}$$

For the first integral on the right of (28), we have the addition theorem for the Bessel function $J_0(x)$ [11]

$$J_0(\tau\sqrt{\ldots}) = \sum_{m=-\infty}^{\infty} J_m(\tau r) J_m(\tau\rho) e^{im(\varphi - \theta)}$$

an expansion which is uniformly and absolutely convergent for all real values of the arguments. Hence

$$\int_0^\infty J_0(\tau\sqrt{\ldots}) \frac{2\tau\, d\tau}{\tau^2 + \beta^2 - k^2} = 2\sum_{m}^{} e^{im(\varphi - \theta)} \int_0^\infty \frac{J_m(\tau r) J_m(\tau\rho)\tau\, d\tau}{\tau^2 + \beta^2 - k^2} \tag{28a}$$

Let us define

$$\gamma^2 = \beta^2 - k^2.\ \beta > k,$$
$$= k^2 - \beta^2,\ \beta < k.$$

Then the integral in (28a) has the value [11, p. 429]

$$\left.\begin{aligned} &I_m(\gamma r) K_m(\gamma\rho),\ \rho > r \\ &I_m(\gamma\rho) K_m(\gamma r),\ \rho < r \end{aligned}\right\} \beta > k$$

$$\left.\begin{aligned} &\frac{\pi i}{2} J_m(\gamma r) H_m^{(1)}(\gamma\rho),\ \rho > r \\ &\frac{\pi i}{2} J_m(\gamma\rho) H_m^{(1)}(\gamma r),\ \rho < r \end{aligned}\right\} \beta < k \tag{29}$$

where $J_m(x)$, $H_m(x)$, $I_m(x)$, $K_m(x)$ are the ordinary Bessel function, Hankel function, modified Bessel function, and modified Hankel function, respectively, all of order m. Let $F_m(\gamma r,\gamma\rho)$ denote the value of the integral. Then we have for (28)

$$\int_0^\infty \frac{e^{ikR}}{R} e^{i\beta\zeta}\, d\zeta = 2e^{i\beta z} \sum_m e^{im(\phi-\theta)} F_m(\gamma r,\gamma\rho) \tag{30}$$

$$+\int_0^\infty J_0(\tau\sqrt{\ldots})\, \frac{e^{-z\sqrt{\tau^2-k^2}}\,\tau\, d\tau}{(i\beta+\sqrt{\tau^2-k^2})\sqrt{\tau^2-k^2}}$$

Let us notice, in (30), that the integral on the right-hand side comes from the fact that the beam "begins" at $z = 0$. If the domain of the densities had been taken to be the unbounded cylindrical domain $0\leq r\leq B$, $-\infty<z<\infty$, then (24a) would have been replaced by

$$\int_{-\infty}^\infty \frac{e^{ikR}}{R} e^{i\beta\zeta}\, d\zeta$$

and consequently the integral in (27) with respect to x would also have extended over $-\infty<x<\infty$, leaving us with just the series part of the right-hand side of (30). The mentioned integral thus gives that part of the field arising from the fact that our beam model is discontinuous at $z = 0$: for $z<0$, the medium is free of charge, but for $z>0$ it is not. We show in the Appendix that this part of the integral is vanishingly small except *near* $z = 0$, and tends to zero as $z\to\infty$, uniformly over the beam cross-section. Thus for sufficiently large values of z (30) is, approximately,

$$\int_0^\infty \frac{e^{ikR}}{R} e^{i\beta\zeta}\, d\zeta = 2e^{i\beta\zeta} \sum_{m=-\infty}^{\infty} e^{im(\phi-\theta)} F_m(\gamma r,\gamma\rho) \tag{31}$$

Then (24) becomes

$$I(r,\varphi,z,t) = 2e^{i(\beta z - \omega t)} \sum_{m=-\infty}^{\infty} e^{im\varphi} \int_0^{2\pi} e^{i(n-m)\theta} d\theta \int_0^B A(\rho) F_m(\gamma r,\gamma\rho)\rho d\rho$$

every term of which vanishes identically except when $m = n$:

$$I(r,\varphi,z,t) = 4\pi e^{i(\beta-\omega t)} e^{in\varphi} \int_0^B A(\rho) F_m(\gamma r,\gamma\rho)\rho d\rho \qquad (32)$$

We therefore have the following result: *let* D: $0 \le r \le B$, $z >> 0$, *and let* $\delta(Q,t) = A(r) e^{in\varphi} e^{i(\beta z-\omega t)}$ *on* D. *Then, if* R *is the distance from* Q $(=\rho,\theta,\zeta)$ *in* D *to an arbitrary point* P $(= r,\varphi,z)$,

$$\int_D \frac{e^{ikR}}{R} \delta(Q,t) d\tau = 4\pi\, e^{in\varphi} e^{i(\beta z-\omega t)} \int_0^B A(\rho) F_n(\gamma r,\gamma\rho)\rho d\rho \qquad (33)$$

where $F_n(\gamma r,\rho\gamma)$ *is given by expressions (29).*

To evaluate the integral in (33) (denote it by $R_n(\gamma r)$), we have two cases, according as β is $>k$ or $<k$. (It is easy to show that the expressions (29a,b) are continuous at $\beta = k$.) What is the physical significance of these two possibilities? We have already remarked that $u = (\mu\epsilon)^{-\frac{1}{2}}$ is the phase velocity of plane waves in an empty unbounded medium of constants μ,ϵ, i.e., a characteristic velocity for the medium itself. The phase velocity v of the elementary wave with phase factor $\exp i(\beta z - \omega t)$ is just ω/β. We thus have

$$\frac{v}{u} \begin{cases} < 1, & \beta > k, \\ > 1, & \beta < k \end{cases}$$

so that in the first case the phase of the elementary beam wave is traveling in the direction $z>0$ with velocity *less* than that for plane waves in the

medium, and in the second case, *greater*. Let us consider the first case, which is relevant to TWAs. There are again two possibilities, according as P lies within (r<B) or without (r>B) the beam. In the former case,

$$R_n(\gamma r) = K_n(\gamma r)\int_0^r A(\rho)I_n(\gamma\rho)\rho d\rho + I_n(\gamma r)\int_r^B A(\rho)K_n(\gamma\rho)\rho d\rho \tag{34}$$

and in the latter

$$R_n(\gamma r) = K_n(\gamma r)\int_0^B A(\rho)I_n(\gamma\rho)\rho d\rho \tag{35}$$

Comparing the expressions (21), (23) and (33), the scalar potential $\Phi(P,t)$ of the charge density wave is given by

$$\Phi(P,t) = \frac{1}{\epsilon} R_n(\gamma r)\, e^{in\varphi}\, e^{i(\beta z - \omega t)} \tag{36a}$$

and the axial component $A(P,t)$ of the vector potential of the current density wave $J = B(r)\, e^{in\varphi}\, e^{i(\beta z - \omega t)}$ is, similarly,

$$A(P,t) = \mu\, S_n(\gamma r)\, e^{in\varphi}\, e^{i(\beta z - \omega t)} \tag{36b}$$

where in (36)

$$\left.\begin{matrix} R_n(\gamma r) \\ S_n(\gamma r) \end{matrix}\right\} = \int_0^B \left.\begin{matrix} A(\rho) \\ B(\rho) \end{matrix}\right\} F_n(\gamma r,\gamma\rho)\rho d\rho \tag{37}$$

Let us now calculate the fields $\overline{E}$ and $\overline{H}$ produced by the densities. The formulas (4) reduce to

$$E_r = -\frac{\partial\Phi}{\partial r},\quad E_\varphi = -\frac{1}{r}\frac{\partial\Phi}{\partial\varphi},\quad E_z = -\frac{\partial\Phi}{\partial z} - \frac{\partial A}{\partial t}$$

$$\mu H_r = \frac{1}{r}\frac{\partial A}{\partial\varphi},\quad \mu H_\varphi = -\frac{\partial A}{\partial r},\quad H_z = 0$$

Applying these to (36), we obtain in cylindrical coordinates

$$\left.\begin{aligned}
E_r &= -\frac{\gamma}{\epsilon_n} R_n'(\gamma r) \\
E_\varphi &= -\frac{in}{r} R_n(\gamma r) \\
E_z &= -\frac{i\beta}{\epsilon_n} R_n(\gamma r) + i\omega\mu S_n(\gamma r) \\
H_r &= in S_n(\gamma r) \\
H_\varphi &= -\gamma S_n'(\gamma r) \\
H_z &= 0
\end{aligned}\right\} \times e^{in\varphi} e^{i(\beta z - \omega t)} \tag{38}$$

(in which the prime means derivative with respect to γr). It follows that *the field vectors* $\overline{E}$ *and* $\overline{H}$ *associated with densities given by the sum of an arbitrary finite number of elementary densities, is just the corresponding sum of expressions (38).* Let us remark also that potentials (36) are particular solutions of the non-homogeneous equations (2) and (3) for the elementary densities.

We conclude with an example. Let $f(z)$ be the even function of period L defined by (Fig. 4)

$$\left.\begin{aligned}
f(z) &= 1 - \frac{2z}{d}, \quad 0 < z < \frac{d}{2}, \\
&= 0, \quad \frac{d}{2} < z < \frac{L}{2},
\end{aligned}\right\} \quad 0 < d < L \tag{39}$$

$$f(-z) = f(z), \; f(z + L) = f(z)$$

Computation gives

$$f(z) = \frac{d}{2L} + \frac{d}{L} \sum_{m=1}^{\infty} \left(\frac{\sin\theta_m}{\theta_m} \right)^2 \cos \frac{2\pi m}{L} z$$

$$= \frac{d}{2L} \sum_{M=-\infty}^{\infty} \left(\frac{\sin\theta_m}{\theta_m} \right)^2 e^{i \frac{2m\pi}{L} z}, \quad \theta_m = \frac{m\pi d}{2L}$$

Then the expression

$$\frac{d}{2L} \sum_{-\infty}^{\infty} \left(\frac{\sin\theta_m}{\theta_m} \right)^2 e^{i \frac{2m\pi}{L}(z - v_0 t)}$$

represents a wave traveling in the direction $z>0$ with constant velocity v_0 ($<u$) and which coincides with the function (39) at $t = 0$. Let us further modify the expression by introducing a dependence on the radius, say

$$1 - \left| \frac{r}{B} \right|^{|m|}$$

for the m—th harmonic. Then

$$\delta(r,\varphi,z,t) = -\frac{\delta_0 d}{2L} \sum_{m=-\infty}^{\infty} \left(\frac{\sin\theta_m}{\theta_m} \right)^2 \left(1 - \left| \frac{r}{B} \right|^{|m|} \right) e^{i \frac{2m\pi}{L}(z - v_0 t)} \tag{40}$$

($0 \le r \le B$) where $\delta_0 = \text{const.} > 0$, is a density of the sort considered above, and $J = \delta v_0$ the corresponding current density.
Comparing (40) with (33) and subsequent expressions, we set

$$n = 0, \beta = \frac{2m\pi}{L}, \omega_m = v_0\beta, A(r) \sim 1 - \left| \frac{r}{B} \right|^{|m|}$$

Let us compute, for instance, E_z at points outside the beam. We have

$$R_0(\gamma r) = K_0(\gamma r) \int_0^B \left(1 - \left(\frac{\rho}{B} \right)^{|m|} \right) I_0(\gamma\rho)\rho d\rho$$

$$= F_0(\gamma B) K_0(\gamma r)$$

Then (38) gives us

$$E_z = \frac{\delta_0 d}{2L} \sum_{m=-\infty}^{\infty} (i\omega\mu v_0 - \frac{i\beta_m}{\epsilon})$$

$$\left(\frac{\sin\theta_m}{\theta_m}\right)^2 F_O(\gamma_m B) K_O(\gamma_m r) e^{i\frac{2m\pi}{L}(z-v_0 t)}$$

(where $\gamma_m^2 = \beta_m^2 - k_m^2 = \left(\frac{2m\pi}{L}\right)^2 (1 - \left(\frac{v_0}{u}\right)^2)$,), in which we have neglected the static part (m = O) of the field.

In a subsequent paper, we shall take up the question of how the densities may be chosen in order to represent phenomena in TWAs.

APPENDIX

We consider the second term on the right-hand side of (30), *viz.*, the improper integral

$$T = \int_O^\infty J_0(M\tau) \frac{e^{-z\sqrt{\tau^2 - k^2}} \tau d\tau}{(i\beta + \sqrt{\tau^2 - k^2})\sqrt{\tau^2 - k^2}}$$

where $M = \sqrt{\tau^2 + \rho^2 - 2r\rho\cos(\theta - \varphi)}$, in which β and k are fixed non-zero real numbers $\beta > k$. Near $\tau = k$, the integrand is proportional to $(\tau - k)^{-\frac{1}{2}}$, which is integrable. In fact we can write

$$T = \int_O^k + \int_k^\infty$$

In the first integral, set $\tau = k\sin x$, in the second, $\tau = k\cosh x$. Then

$$T = -\int_O^{\pi/2} J_0(Mk\sin x) e^{-izk\sin x} \frac{k \sin x \, dx}{\beta + k\cos x}$$

$$+\int_0^\infty J_0(Mk\cosh x)\,e^{-zk\sinh x}\,\frac{k\cosh x\,dx}{i\beta + k\sinh x}$$

$$= T_1 + T_2$$

and since $\beta > k$, the first integrand is continuous. Consider the first integral T_1; we have the identity

$$e^{-izk\sin x} = \sum_{-\infty}^{\infty} i^{-n} J_n(kz)\,e^{inx}$$

the right-hand side of which is uniformly and absolutely convergent for every real z. Then

$$-T_1 = \sum_{-\infty}^{\infty} i^{-n} J_n(kz) \int_0^{\pi/2} J_0(Mk\sin x)\,e^{inx}\,\frac{k\sin x\,dx}{\beta + k\cos x}$$

and

$$|T_1| \le k \sum_{-\infty}^{\infty} J_n(kz) \int_0^{\pi/2} |J_0(Mk\sin x|\,\frac{|\sin x|\,dx}{|\beta + k\cos x|}$$

Since $|J_0(Mk\sin x| \le 1$, we have

$$|T_1| \le \frac{\pi}{2}\,\frac{k}{\beta - k} \sum_{-\infty}^{\infty} |J_n(kz)|$$

But every term of the last series tends to zero as $z \to \infty$.

For the second integral T_2, we have

$$|T_2| \le \int_0^\infty e^{-zk\sinh x}\,\frac{k\cosh x\,dx}{\sqrt{\beta^2 + k^2\sinh^2 x}}$$

If we set u = ksinh x, the last integral becomes

$$\int_0^\infty e^{-uz} \frac{du}{\sqrt{\beta^2 + u^2}} = J_0(\beta z)$$

which also vanishes as z→oo.

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Manuscript received July 6, 1967.

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Volume 61
No. 4
1968

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(01335—9-68)

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TRANSACTIONS

OF THE

ILLINOIS STATE ACADEMY OF SCIENCE

VOLUME 61 - 1968 No. 4

ILLINOIS STATE ACADEMY OF SCIENCE

AFFILIATED WITH THE

ILLINOIS STATE MUSEUM DIVISION

SPRINGFIELD, ILLINOIS

PRINTED BY AUTHORITY OF THE STATE OF ILLINOIS

SAMUEL H. SHAPIRO, *Governor*

December 1, 1968

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CERTAIN ASPECTS OF HUMAN CHORIONIC GONADOTROPIN (HCG) ON RAT SERUM PROTEINS

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ABSTRACT. — A study was conducted to determine the influence of human chorionic gonadotropin (HCG) on the fractionated serum proteins of injected virgin female white rats.

Comparisons of electropherograms showed an increase in the relative percentages of alpha and beta globulins, but no apparent changes could be detected in the gamma globulins. Concurrent decreases in the serum albumin fractions were accredited to the changes in globulin percentages.

Since the discovery of human chorionic gonadotropin (HCG) by Friedman in 1929, the hormone has been studied in the light of its primary function — that of a luteinizing hormone, aiding in the production of progesterone in the early stages. In 1961, Nagy *et al.* found that HCG was bound to the beta globulin fraction in the serum of pregnant women. This same fraction binding existed even when crystalline HCG was mixed with serum *in vitro*. The researchers concluded that the hormone was bound in a loose and passive manner, without the involvement of binding or coupling factors.

The presence of such a hormone has not, as yet, been found in the rat. An experiment was undertaken to determine the effects of HCG administration on serum proteins.

PROCEDURE

Twenty-four Sprague-Dawley rats were used in the experiment. All animals were virgin females, ranging from 175-200 grams and 3-4 months of age.

Twelve of the 24 females received 20 I.U. of HCG daily for 4 consecutive days. The hormone was purchased from Rugby Laboratories and diluted with Rugby diluent to a solution containing 40 I.U. per ml. Following the administration of HCG, blood serum samples were prepared for electrophoresis. Serum samples were procured from control females, that did not receive HCG injections, for electropherogram comparison of serum protein fractions.

Electrophoresis was conducted on all serum samples by the use of equipment manufactured by the Gelman Instrument Company. The procedure employed was described in the Gelman Manual No. 70176-A. Quantitation of each electropherogram was accomplished through the use of a Densicord Densitometer, produced by the Photovolt Corporation, New York.

The densitometric tracings were demarcated, cut out, and weighed on a Mettler Balance. Values were recorded in terms of each fraction's densitometric tracing paper weight as a percentage of the total proteins separated by electrophoresis. The serum fractions were identified by the electropherograms shown in the Gelman Manual.

At the completion of the HCG treatment, all treated and control animals were sacrificed. The ovaries and uteri were extirpated and weighed. Values were recorded in terms of each organ's percent of the animal's total body weight.

Results and Discussion

A comparison of the ovarian and uterine weight percentage of total body weight, between treated and control animals, are shown in Table 1.

Table 1.—A comparison of ovarian and uterine weights of control and HCG injected rats.

Organ	Control mean wt[a]	HCG mean wt
ovaries	.0351	.0648**
uterus	.1018	.1588**

[a] Values presented are the means of each organ's percentage of the total body weight.
** $P < .01$

The mean ovarian percentage weights were nearly double that of their respective control animals, while the mean uterine percentage weights showed greater than a 50 percent increase over the control group. These particular results demonstrated the biological activity present under the hormonal influence and supports possible luteinizing effects of HCG.

Data, resulting from animals treated for 4 days with 20 I.U. HCG are shown in Table 2 and Figures 1 and 2. Each of the alpha and beta globulin percentages increased significantly when compared with control serum samples. Significant decline in serum albumin was noted, whereas serum gamma globulin did not change.

These observed changes in serum fractions possibly reflect an effect of HCG on the liver site of the plasma

Table 2.—Comparison of rat serum protein fractions[a] before and after the injection of HCG

Serum protein fraction	Control animal serum	Serum from HCG injected animals
Albumin	57.8	54.2**
Alpha—1 globulin	3.9	4.6*
Alpha—2 globulin	4.3	5.0*
Beta—1 globulin	5.6	6.6*
Beta—2 globulin	12.3	14.9**
Gamma globulin	15.9	15.7

[a] Values represented as a percentage of the total proteins separated.
* $P < .05$
** $P < .01$

globulins involved. According to Harper (1965), fats in the blood are bound to proteins to render them soluble and facilitate transport. These lipoproteins migrate to the alpha and beta regions of the plasma globulins. Any increase in mobilization of stored fats would cause increased plasma fats and a concurrent increase in their binding proteins. The protein — specific stain used in this procedure would detect the relative amounts of these lipoproteins present. Also, it must be considered that other hormones such as thyroxin, insulin, and hormones of the adenohypophysis bind with the alpha and beta globulins (Harper, 1965). An effect of HCG on any endocrine gland to increase its output would likewise show an increase in its respective carrier proteins in the blood.

Upon examination of the electropherograms after treatment, it was noted that an additional bind had appeared in the beta 2 area. This band change is shown in Figure 2. In the light of the report by Nagy *et al.*, 1961, who stated that HCG activity was detected in association

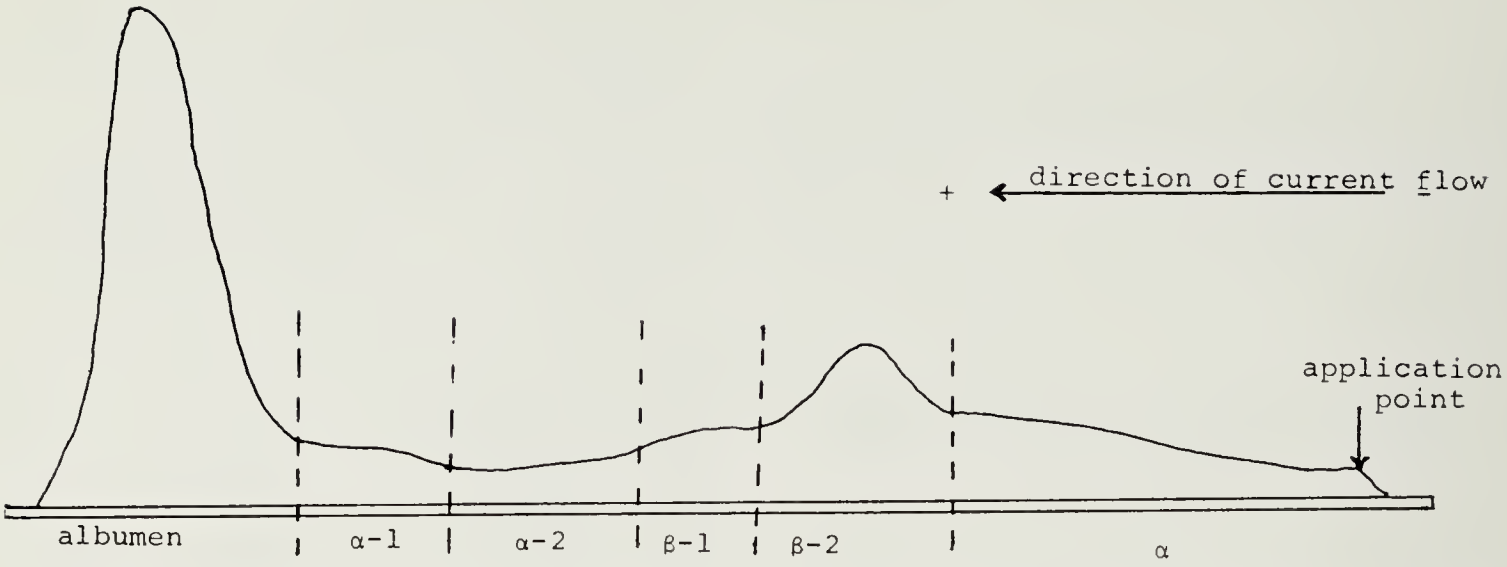

FIGURE 1.—Densitometric tracings of an electropherogram made from serum protein samples from rats that did not receive HCG injection.

with the beta globulin fraction, this extra densitometric peak could represent a reflection of the HCG binding at that location.

The percentage decline in albumin may be accounted for by consideration that these values were expressed in percentage of the total protein separated and therefore albumin changed very little in absolute concentration as compared with changes observed in alpha and beta globulin.

The lack of response in gamma globulin fraction was certainly noteworthy. Since HCG is proteinous and foreign to rat's tissues, elicitation of an antibody response might have been expected, with an increase in the gamma globuiin fraction following treatment.

SUMMARY AND CONCLUSION

From the experiment conducted on the influence of HCG on rat serum proteins, the following may be summarized:

a) HCG affected ovarian and uterine activity in the female rat.

b) Injection of HCG had the effect of increasing the relative percentages of alpha and beta globulins, but no apparent effect on the gamma globulin fraction.

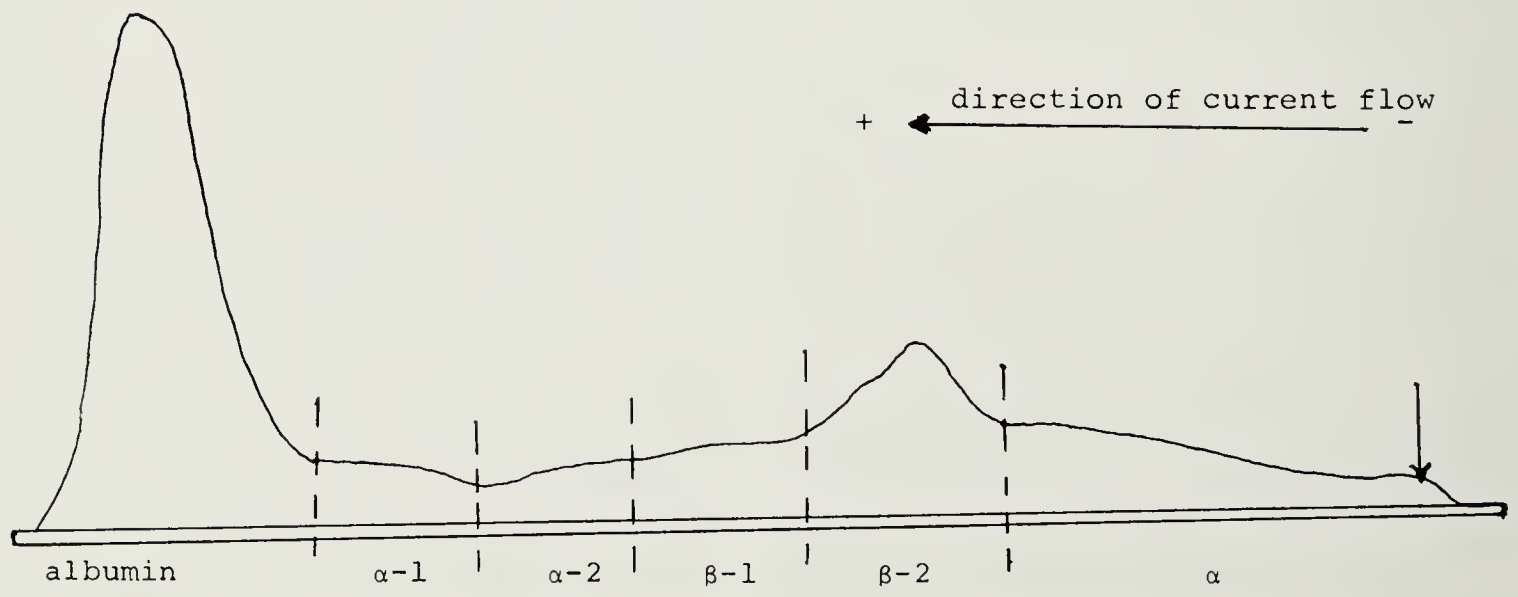

FIGURE 2.—Densitometric tracings of an electropherogram made from serum protein samples from rats receiving HCG injections.

c) Decreases in the albumin fraction percentage were at least accounted for by concurrent changes in globulin percentages.

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Manuscript received December 7, 1967.

FOREST TYPES OF LUSK CREEK IN POPE COUNTY, ILLINOIS

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ABSTRACT. — Forest types on the valley bottoms and on lower to mid slopes were described from 1562 trees sampled by the wedge prism method. Two distinct communities were consistently evident. On terraces adjacent to the major drainages sugar maple, beech locally, and tulip trees predominated. White oak predominated on the mid slopes. Associated species included red oak, oval pignut hickory, and black oak. The ecological structure and associated shrub and herbaceous species were characteristic for each community. Species typical of bottomland habitats along the Mississippi River were conspicuously absent. The hypothesis is presented that sedimentation during post-glacial events on the Ohio River eliminated the bottomland habitat characteristic of southwestern Illinois, and greatly reduced the lower slope habitat on the middle sector of Lusk Creek.

Lusk Creek is the major drainage of Pope County. It originates in the Shawnee Hills at 800 feet elevation. The drainage basin, 5 miles wide by 15 miles long, empties into the Ohio River, 310 feet meanpool elevation, at Golconda. In the hill portion the courses of the main stream and its tributaries follow geologic faults to a marked degree. Canyon and rock walls are prominent in Pennsylvanian sandstone formations. Relatively small areas of shale and limestone are found.

Several vegetation types are found in the middle Lusk Creek drainage. The broad ridges and broad valley bottoms which were cleared for farming are today either still being farmed or are occupied by successional vegetation. On the uplands this is largely little bluestem (*Andropogon scoparius*) with invading tree species such as winged elm, flowering dogwood, and red cedar. Numerous pine plantations are present. Bottomland old fields may have young canopy forests of box elder or of mixtures including box elder, river birch, sycamore, and tulip tree. A conspicuous herbaceous layer is usually present.

The remaining forest vegetation can be divided into several types. Along the larger streams river birch is a conspicuous species where gravel bars have been colonized by trees. Sycamore occurs along both the larger and the smaller intermittent streams.

The valley bottoms adjacent to larger streams support a well developed forest type, including sugar maple, beech, tulip tree, hickories, and black gum, which will be described in detail later in the paper, as will those of the lower and mid

slopes. The slope forests are dominated by white oak and other oaks and hickories. The upper slopes and ridges support oak-hickory forests in which black oak, scarlet oak, post oak, and oval pignut hickory may be important species. Understory trees such as ironwood, dogwood, and winged elm are characteristic of many slope forests.

The Illinoisan glaciation reached to within about 10 miles of the study area. Geologic influences of Wisconsin glaciation are found in loess deposits and in the levels of valley sediments. Surface soil materials are silty, usually with little sand, and little clay. On the slopes rock is often present, and local areas may have numerous large boulders or outcrops.

A marked change in slope occurs from the valley sides to level areas which may extend a few hundred feet in width adjacent to a stream. The active stream channel is entrenched 6 to 12 feet below the level valley bottoms. Usually silt predominates in the entrenchment wall, with sand variable and generally low in amount. No areas with substantial amounts of clay were found. Above the 400-foot contour, bedding in the stream bank revealed occasional areas with extensive gravel.

The channel areas between extensive pools which persist through the summer are predominantly gravel. Thickets of river birch, with occasional deciduous holly (*Ilex decidua*), may be found on these gravel areas. Usually the channel splits to form island-like segments of the flat valley bottom below the confluence of smaller drainages from the adjacent hills. Where the larger streams meet there may be broader, alluvial plains more than 1000 feet across which have been cleared for agriculture.

Climatically the county is characterized by hot summers (average July temperature over 78°F.), moderate winters (average January temperature over 34°F.), and generally well-distributed precipitation with an average at Golconda on the Ohio River of 45 inches (Page, 1949). The extent of topoclimatic variation in the hill country which includes steep canyons and narrow valleys is not known.

Materials and Methods

The study areas selected extended 6 to 10 miles upstream from a projected high dam site at 340 feet stream elevation in Section 32, Township 12 S, Range 6 E, Pope County, southeastern Illinois, approximately midway between Eddyville and Golconda. The estimated lake level of approximately 455 feet above sea level formed an upper limit for study areas both upstream and on hill slopes. Other limits were the occurrence of well-developed forests of adequate size for sampling. These became restricted as the valleys narrowed going upstream.

Preliminary forest evaluations were made from topographic maps and aerial photographs. Most of the area was also inspected by foot. A sight list of the trees was compiled for each area visited. Plotless estimates of basal area using point sampling with a wedge prism, basal area factor 10, (Grosenbaugh 1952, 1958) were carried out in selected stands approximately 100 feet or more across and several hundred feet in extent. Using an optical system to determine an exclusion angle in selecting trees to tally, the probability of tree selection is proportional to tree basal area. Trees less than 3.6 inches in diameter (4-inch class) were excluded. Sampling points were usually 100 feet apart. In areas of exceptionally large trees, greater distances were used. The valley bottom areas with good timber stands were sampled rather thoroughly. A total of 359 trees from 4 major areas were tallied in the Little Lusk Creek drainage, and 585 trees from 5 areas in that part of the Lusk Creek drainage

upstream from the projected dam site. Representative areas of good stand development on the slopes along Lusk Creek were sampled for a total of 618 trees on 6 major areas. The larger trees of the various species were measured to indicate relative sizes.

Observations were made of the ecological structure of the forest communities on the two physiographic sites. A cover estimate was made for the canopy, understory, shrub, and herbaceous strata or layers. Dominant species in the several layers were listed. Evidences of past disturbances such as stumps were recorded. In each point sample all dead trees were tallied, and where possible species identification made.

Slopes ranging from 7 to 26 degrees were chosen. The average count of trees for each location using the wedge prism was multiplied by the secant of the average angle of slope perpendicular to the contour. The valley bottoms were essentially flat and no correction was used. Steep slopes were not sampled, in part because of the greater correction necessary in using the wedge prism. The tree cover on steep slopes also showed a greater mixture of species from the forest types usually associated with ridges or valley bottoms than did the less steep slopes.

Edaphic and physiographic features were observed. Primary interest centered on the lay of the land with respect to the creeks and the hills. The nature of the surface soil materials, presence of rock and rock outcrops, nature of the drainage features, and the type of subsurface materials exposed along streams were noted.

Results and Discussion

A total of 42 tree species were tallied for the point samples. (Table 1). The species had characteristic dominance relationships in the several habitats, as will be discussed later. All the dominant species were those found widespread throughout southern Illinois in the appropriate habitats. Other tree species recorded include redbud (*Cercis canadensis*), pawpaw (*Asimina triloba*), persimmon (*Diospyros virginiana*), mulberry (*Morus rubra*), cottonwood (*Populus deltoides*), and willow (*Salix sp.*). Green ash (*Fraxinus pensylvanica*) was not separated out.

The average total basal areas for the three major areas — valley bottoms along Lusk Creek, along its tributary, Little Lusk Creek, and on slopes along Lusk Creek — showed a decrease in basal area from 106 to 97 to 86 square feet per acre. Four species were tallied on the valley bottoms and not on the slopes. Twelve were tallied on the valley bottom for Lusk Creek, and not on Little Lusk. Eight species were tallied for the slope points and not for the valley bottoms. The slopes had quite different proportionate basal area values for the several species which occurred on both types of site.

A clear-cut dominance of sugar maple, and of beech, was evident for the valley bottoms. Together they constituted 47% and 44% of the total basal area for the Lusk and Little Lusk areas respectively. With the addition of tulip tree these values became 69% and 53% respectively. The basal area for tulip tree, however, approximates about half that of the beech. Species with intermediate values for basal area included bitternut and oval pignut hickories, black gum, and ash. To the extent that species identifications were possible, the ash appeared to be *Fraxinus americana*, white ash. In order of basal areas, the 10 leading species on the 3 major study areas were:

Lusk Creek Terrace

1. Sugar maple
2. Beech
3. Tulip tree
4. Bitternut hickory
5. Black gum

6. Oval pignut hickory
7. Ash
8. Shagbark hickory
9. Sycamore
10. American elm

Little Lusk Terrace

1. Sugar maple
2. Beech
3. Tulip tree
4. Oval pignut hickory
5. Bitternut hickory
6. Black gum
7. Ash
8. Red elm
9. Black walnut
10. Red oak

Lusk Creek Slopes

1. White oak
2. Red oak
3. Oval pignut hickory
4. Black oak
5. Sugar maple
6. Tulip tree
7. Red elm
8. Black gum
9. Chinquapin oak
10. Shumard oak

White oak constituted a third of the basal area for the slope forest communities. The next 4 species constituted approximately 10% each so that a more mixed community with shared dominance was characteristic of these areas. Species more characteristic of the valley bottoms and found on the slopes were often associated with small drainage depressions on the slopes.

Dead trees included in the wedge prism points were tallied separately. They constituted 6 square feet or 6% additional basal area on the terraces and 8 square feet or 9% additional on the slopes. About half of the dead trees were identified to species or to genus. Of these trees the oaks (on the slopes) amounted to a third, the hickories a quarter, the elms a tenth, beech and sassafras less than a tenth, with 8 other species identified. Thus no striking disproportionate mortality to the numbers of live trees was evident, other than perhaps a low value for sugar maple.

The terrace and the slope communities were distinctive in ecological structure. In the white oak community on the lower and mid slopes the canopy was not fully closed, the understory of dogwood, hop hornbeam, winged elm, as well as reproduction of canopy species, was well developed, and the shrub and herbaceous layers were consistently present and generally not closed. In contrast, the sugar maple communities of the terraces had a more closed canopy, a poorly-developed understory tree stratum, and two well-developed often alternate lower story layers. In many areas, particularly along the wider terraces in the lower reaches of the stream, a pronounced herbaceous layer was essentially continuous, with very few woody plants in evidence. At the time of the major study in mid September white snakeroot (*Eupatorium rugosum*) was conspicuous in a layer about 3 feet tall. Other areas had a continuous shrub layer with bladdernut (*Staphylea trifolia*) often predominant. Spicebush (*Lindera benzoin*) was a common associate. Exceptional areas such as Martha's Woods bordered on three sides by a bend of Little Lusk Creek (Sugar Camp) had both a rich herbaceous layer and groupings of shrubs present. This area appears

to be essentially virgin, with no stumps, large trees, and a basal area of 120 square feet.

Interesting and rare herbs of the sugar maple communities included black cohosh (*Cimicifuga cordifolia*), richweed (*Collinsonia canadensis*), and valerian (*Valeriana pauciflora*). Frequently found were wild ginger (*Asarum reflexum*), baneberry (*Actea alba*) and Indian pipe (*Monotropa uniflora*). The rattlesnake plantain (*Goodyera pubescens*), so rare in Illinois, puttyroot (*Aplectrum hyemale*), and other orchids were found in the slope communities.

Examination of the tree data by local study areas reveals interesting variations. Sugar maple was generally distributed on the valley bottoms. Beech, in contrast, predominated in some valley bottom areas and was completely absent from a few others. The frequency of basswood, which has a limited distribution in southern Illinois, increased on going downstream. It generally occurred on the terrace next to the entrenched channel. Buckeye showed a somewhat similar pattern.

Several tree species showed noteworthy phytogeographic relationships. River birch, so common along the channel of Lusk Creek, is missing from the Ozark limestone region of southwestern Illinois, as are three upland oaks — Spanish, shingle, and scarlet. In contrast, the percentage of shagbark hickory at Lusk Creek was low compared to many areas of southern Illinois. The hickories were of taxonomic interest. Their variations exceeded that found in several other areas of southern Illinois and appeared to represent, in part at least, possible crossing between *Carya cordiformis* and another species, perhaps *C. ovalis*.

Bottomland species not found in the Lusk Creek study areas were silver maple (*Acer saccharinum*), generally found along streams, swamp white, cherrybark, overcup, swamp chestnut, and pin oaks (*Q. bicolor*), *Q. falcata* var. *pagodaefolia, Q. lyrata, Q. michauxii*, and *Q. palustris*) and other lowland species of southern Illinois such as pecan (*Carya illinoensis*), sugarberry (*Celtis laevigata*) and Kentucky coffee tree (*Gymnocladus dioicus*). The absence of these species, the occurrence of very few honey locust and only one sweet gum, and the widespread occurrence of species such as sugar maple, butternut, black walnut, tulip tree, and red oak strongly support the concept of the valley bottom sites along the streams being terraces. Phytogeographically they are quite unlike floodplains or bottomlands as usually considered.

An overall view of these ecological relationships in the study areas suggests that the valley bottoms (terraces) are high enough above the creek not to experience substantial or prolonged flooding. External, and internal, drainage is sufficiently good to explain the absence of typical bottomland species and the dominance of sugar maple and its ecological associates. Internal drainage may be somewhat limiting, however, and govern the local importance of beech versus sugar maple in several of the study areas. Fritts and Holowaychuk (1959) demonstrated a greater sensitivity of sugar maple than beech to impeded drainage in central Ohio. The distribution of basswood, which

has been considered to require still better drainage than beech or sugar maple (U. S. Dept. Agric., 1965), supports the concept of drainage as a variable on the terraces. The highest basal area (200 square feet) was found for a terrace "island" between two channels on Lusk Creek. Both basswood and buckeye occurred on the "island".

Quaternary events have greatly affected the unglaciated lower Ohio River valley and its tributaries (Ray, 1963). These events include alluviation and loess deposition. Ray (1967) states:

> "Lusk Creek, like others tributary to the Ohio River main stem, has a well-developed terrace in its lower valley. The terrace is underlain by clayey silt, blue-gray where unoxidized, and calcareous below a surficial leached zone of some 4, plus or minus, feet. In the Lusk Creek valley the terrace level is roughly at an altitude of 350 feet above sea level. It is genetically related to the Tazewell outwash valley train in the Ohio Valley and was developed about 19,000 years, plus or minus, before the present."

Detailed studies on the stream morphology, including terraces, of Lusk Creek are in progress by Terry L. Dyroff, a graduate student working with Dr. Stanley E. Harris, Jr. in the Southern Illinois University Geology Department. My present interpretation based on vegetational characteristics and geomorphology is that the type of terrace found in Section 32, elevation 340 feet, extends upstream above the bridge on the Eddyville-Golconda road and roughly to the confluence of Lusk and Little Lusk Creeks, or an elevation of approximately 380 feet. As Ray states, the older floodplain deposits of Lusk Creek contemporaneous with the lacustrine deposits may extend to an altitude of 380 to 400 feet. The low gradient of the stream below this level together with more prominent ponding and bars rather than bedrock in the "channel" support this view. The recurring narrow portions of the valley walls could further have been associated with extensive ponding of the creek at some earlier date, and contributed to local terrace formation.

In comparing the vegetational picture at Lusk Creek with that in several other areas of southern Illinois (Voigt and Mohlenbrock, 1964; Ashby and Kelting, 1963), not only is the bottomland type missing, but a ravine and lower slope type (red oak, tulip tree) is much less evident. If the valley bottoms along Lusk Creek are terraces filled in against the slope to cover the lower slopes on which red oak and tulip tree have their best growth and greatest occurrence, the juxtaposition of the sugar maple community and the white oak community is more understandable.

The vegetation of the Southern Illinois University Pine Hills Field Station in the Ozarks of southwestern Illinois adjacent to the Mississippi River shows interesting contrasts to that at Lusk Creek. There a well-developed slope vegetation (red oak, black gum, beech, tulip tree community) contacts a well-developed bottomland community (sweet gum, cherrybark oak, sugarberry, pin oak, etc.). The sugar maple community, which was recognized, was not sufficiently represented to be mapped and

discussed. These contrasts in vegetation relate to fundamental variations in habitat.

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Manuscript received January 22, 1968.

TABLE 1.—Basal areas and largest diameters recorded for species tallied in the wedge prism points for the three major study areas, the Lusk Creek valley bottom or terrace, Little Lusk Creek terrace, and Lusk Creek slopes.

Species	Lusk	Little L.	Lusk	DBH
	Terrace		Slope	
	Basal Area (square feet)			(inches)
Boxelder (*Acer negundo*)	0.5	.....	.....	15.7
Red maple (*A. rubrum*)	0.9	.....	.....	28.5
Sugar m. (*A. saccharum*)	27.6	22.7	8.2	28.5
Buckeye (*Aesculus glabra*)	0.5	0.5	.....	14.9
River birch (*Betula nigra*)	0.9	.....	.....	33.3
Hornbeam (*Carpinus caroliniana*)	0.4	0.5	.....	5.0
Bitternut h. (*Carya cordiformis*)	5.6	7.8	1.2	28.4
Pignut hickory (*C. glabra*)	0.5	.....	1.0	24.1
Kingnut h. (*C. laciniosa*)	0.2	0.5	0.1	.....
Oval pignut h. (*C. ovalis*)	3.6	8.4	8.6	23.5
Shagbark h. (*C. ovata*)	2.7	1.9	0.4	23.9
Mockernut h. (*C. tomentosa*)	0.2	.....	0.3	28.1
Hackberry (*Celtis occidentalis*)	2.4	1.6	0.1	29.5
Dogwood (*Cornus florida*)	.....	.....	0.1	4.8
Beech (*Fagus grandifolia*)	22.7	19.7	0.8	45.8
Ash (*Fraxinus sp.*)	3.4	4.3	0.4	28.7
Honey locust (*Gleditsia triacanthos*)	0.2	.....	.....	17.9
Butternut (*Juglans cinerea*)	0.4	0.5	0.1	19.0
Black walnut (*J. nigra*)	1.8	2.7	1.0	26.2
Red cedar (*Juniperus virginiana*)	.....	.....	0.1	10.2
Sweet gum (*Liquidambar styraciflua*)	0.2	.....	.....	29.8
Tulip tree (*Liriodendron tulipifera*)	12.7	8.6	2.6	29.5
Black gum (*Nyssa sylvatica*)	4.2	5.1	2.2	22.9
Hop hornbeam (*Ostrya virginiana*)	.....	.....	0.3	5.0
Sycamore (*Platanus occidentalis*)	2.7	0.5	0.4	33.5
Black cherry (*Prunus serotina*)	0.4	0.8	.....	9.6
White oak (*Quercus alba*)	1.6	1.1	29.7	28.0
Scarlet o. (*Q. coccinea*)	.....	.....	1.0	15.7
Shingle o. (*Q. imbricaria*)	0.4	.....	.....	.....
Southern red o. (*Q. falcata*)	.....	.....	0.1	22.5
Bur o. (*Q. macrocarpa*)	0.2	.....	0.3	26.4
Blackjack o. (*Q. marilandica*)	.....	.....	0.3	16.1
Chinquapin o. (*Q. muhlenbergii*)	1.1	.....	2.1	30.0
Red o. (*Q. rubra*)	2.0	2.7	9.7	32.2
Shumard o. (*Q. shumardii*)	.....	1.4	1.8	24.6
Post o. (*Q. stellata*)	.....	.....	0.1	12.5
Black o. (*Q. velutina*)	0.4	0.5	8.5	26.9
Sassafras (*Sassafras albidum*)	0.2	.....	0.4	13.5
Basswood (*Tilia americana*)	0.5	.....	.....	17.9
Winged elm (*Ulmus alata*)	.....	.....	0.1	17.0
American e. (*U. americana*)	2.7	0.5	.....	35.8
Slippery e. (*U. rubra*)	2.2	3.8	2.4	27.9
Total Basal Area per acre	106.2	97.0	85.8	
Total No. of Trees	585	359	618	
Total No. of Dead Trees	31	23	54	
Total No. of Points	55	37	72	

KINETICS OF DECOMPOSITION OF BIS(2,6-DIMETHYLPYRIDINE)SILVER(I) NITRATE

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ABSTRACT. — The kinetics of decomposition of solid bis(2,6-dimethylpyridine)silver(I) nitrate has been studied by an isothermal thermogravimetric method. The reaction is first order in complex and has an activation energy of 30.8 ± 2.8 kcal/mole.

Recently the preparation of bis(2,6-dimethylpyridine)silver(I) nitrate was described (House, 1967). In contrast to previous reports (Peard and Pflaum, 1958), it was also demonstrated that the pure complex undergoes complete decomposition at temperatures much below the melting point. Accordingly, this behavior has prompted us to make a kinetic study of the decomposition of this compelx.

EXPERIMENTAL

Bis(2,6-dimethylpyridine)silver(I) nitrate was prepared as previously described (House, 1967).

Kinetic measurements were perfoimed in the following way. Several 0.1 to 0.15 gram samples of the complex were carefully weighed in small pyrex tubes. These sample tubes were then placed in an aluminum holder and the entire assembly suspended in an oil bath at the desired temperature. Constant temperatures were maintained to within about ± 0.2°. At the desired intervals, the sample tubes were removed, carefully cleaned with benzene, and reweighed. The weight loss was determined for each sample and, from the weight loss, the loss of 2,6-dimethylpyridine was determined. This method is essentially an isothermal thermogravimetric method.

RESULTS AND DISCUSSION

For a reaction which is first order in complex, the rate is proportional to the amount of complex, S.

$$-\frac{dS}{dt} = kS \qquad (1)$$

Integration of equation (1) gives

$$\ln \frac{S_0}{S} = kt \qquad (2)$$

where S_0 is the amount of complex at zero time and S is the amount at some later time, t. If W_0 is the weight of ligand present in a weight of complex S_0, equation (2) is equivalent to

$$\ln \frac{W_0}{W} = kt \qquad (3)$$

Now the fraction of 2,6-dimethylpyridine liberated is determined by the amount of the silver complex that is decomposed. If α is the fraction of the original complex decomposed, then

$$\alpha = \frac{W_0 - W}{W_0} \qquad (4)$$

It must be emphasized that in equations (3) and (4) W_0 is the initial weight of 2,6-dimethylpyridine present in the sample of complex and W

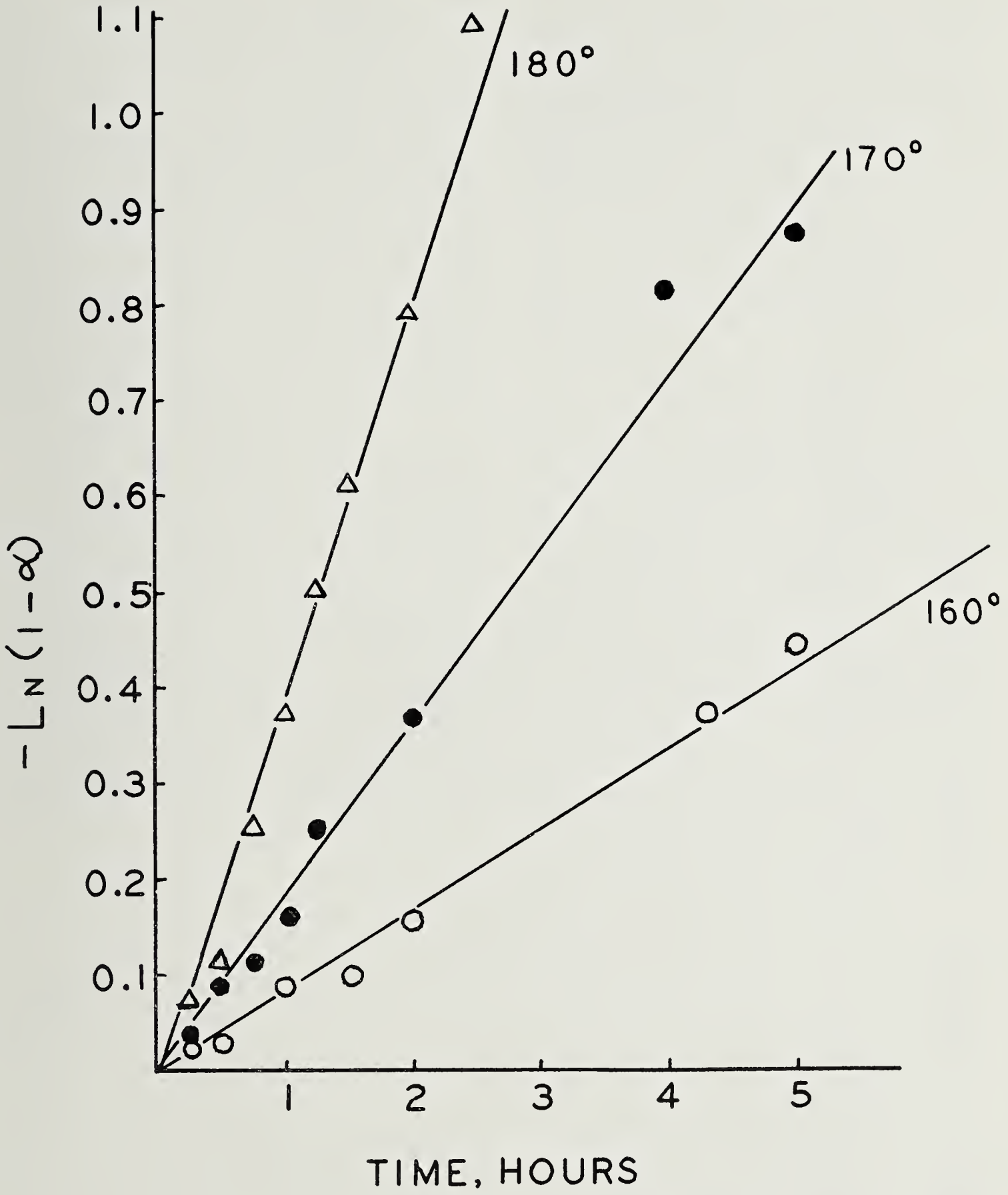

FIGURE 1.—First order rate plots for the thermal decomposition of bis(2,6-dimethylpyridine)silver(I) nitrate.

is the weight lost from that sample. By substituting equation (4) into equation (3), rearrangement leads to

$$-\ln(1-\alpha) = kt \quad (5)$$

Equation (5) is a frequently encountered expression in solid state kinetics (Young, 1966). It is evident that plots of $-\ln(1-\alpha)$ or $\ln(W_0/W)$ against time should be linear. As will be shown later, such plots of the data obtained in this work are linear. If α is small, say less than 0.4 or 0.5,

$$-\ln(1-\alpha) = \alpha \quad (6)$$

and a plot of α against time should

be linear. It has also been found that these plots are linear.

Rate constants were determined from the slopes of the individual rate plots using equation (5). All slopes were determined by a least squares analysis using an IBM 1620 Computer. This procedure was also used to determine the activation energy from the rate constants at different temperatures.

The results of kinetic runs at 160°, 170°, and 180° are shown in Figure 1. It is apparent from Figure 1 that

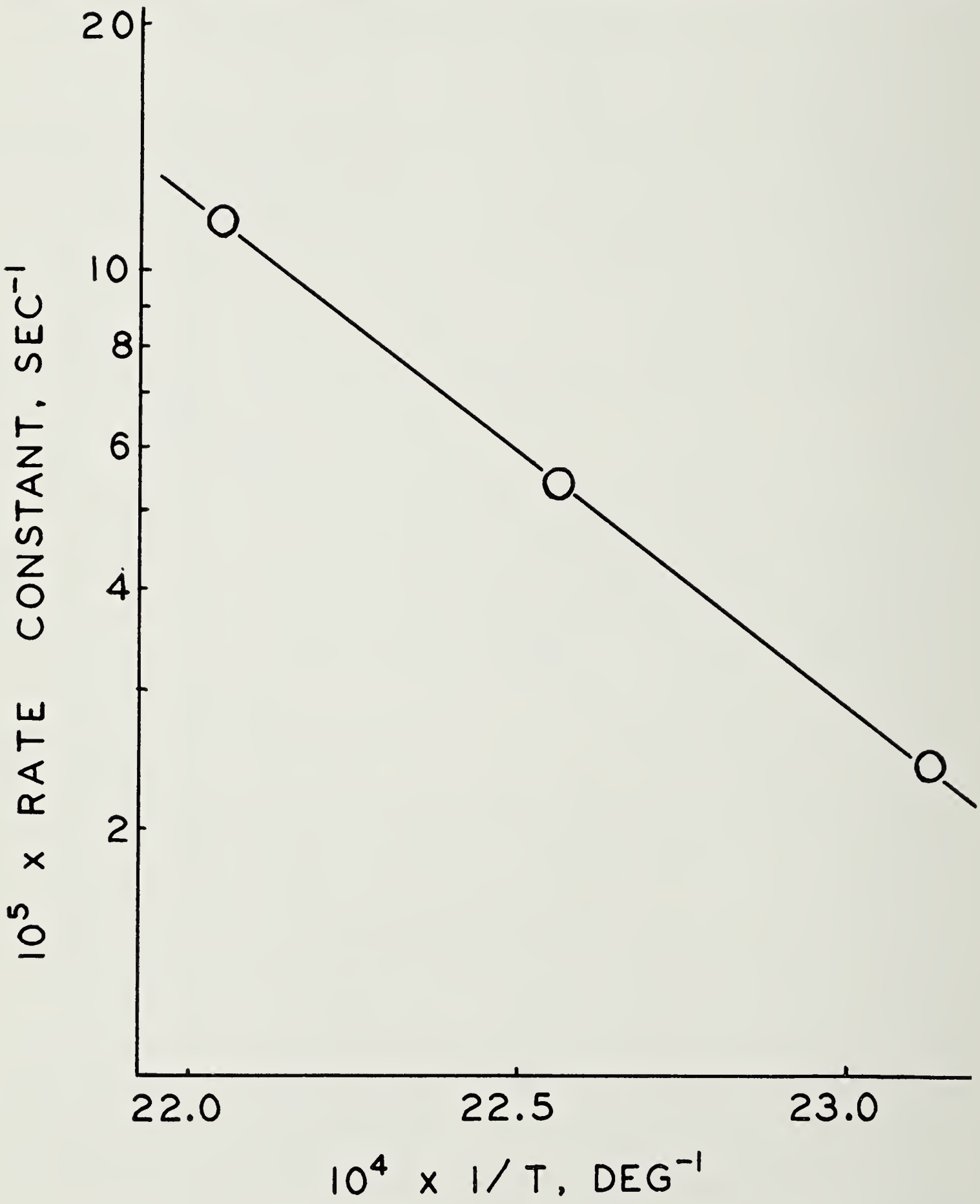

FIGURE 2.—Arrhenius plot for the thermal decomposition of bis(2,6-dimethylpyridine)silver(I) nitrate.

the data fit the first order plot very well. A least squares analysis of the data represented in Figure 1 gives first order rate constants of 2.52 x 10^{-5}, 5.22 x 10^{-5}, and 12.7 x 10^{-5} sec^{-1} at 160°, 170°, and 180° respectively. These rate constants are shown graphically in the Arrhenius plot of Figure 2. The slope of this line corresponds to an activation energy of 30.8 kcal/mole. An estimation of the error in this value by the procedure of Benson (1960) indicates the value to be 30.8 $\pm$ 2.8 kcal/mole.

This study does not enable one to say much about the mechanism of thermal decomposition of bis(2,6-dimethylpyridine)silver(I) nitrate. It is extremely unlikely that both ligand molecules are lost simultaneously. Probably one molecule is lost slowly per molecule of complex and the unstable product rapidly decomposed into silver nitrate and the second ligand molecule. A preliminary run using differential scanning calorimetry shows a fairly broad endotherm with two maxima with only a slight temperature separation. This may reflect the stepwise decomposition of this complex.

Acknowledgment

The authors wish to express their appreciation for the cooperation and support of the Division of Computer Services.

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Manuscript received February 5, 1968.

A REVIEW OF ABSOLUTE ASYMMETRIC SYNTHESES BY CIRCULARLY POLARIZED LIGHT

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ABSTRACT. — The paper discusses the use of circularly polarized light for the purpose of absolute asymmetric syntheses. A theoretical section includes mechanistic requirements as well as a quantitative description of the process. Pertinent experiments involving both asymmetric photolysis and asymmetric photosyntheses are reviewed.

An absolute asymmetric synthesis is the production of optically active materials without the intervention of any dissymmetric chemicals. Such a synthesis requires the influence of some dissymmetric physical agent. By convention, and perhaps unfortunately, the phenomenon of fortuitous spontaneous crystallization from a supersaturated racemic modification has also been classified as an absolute asymmetric synthesis. This paper will be confined to a discussion of the use of circularly polarized light as the influencing physical element. Some of the theoretical aspects of this kind of synthesis will be discussed as well as the more significant experiments of this area.

THE BASIS OF ASYMMETRIC PHOTOCHEMISTRY

Haidinger (1847) discovered that in certain spectral regions circularly polarized light of opposite sign is unequally absorbed by quartz crystals (circular dichroism). Later, Cotton (1895) demonstrated circular dichroism in the region of optically active absorption bands. Van't Hoff (1894) predicted that conversely, if circularly polarized light of a given sign is unequally absorbed by the (+) and (—) forms of an optically active material, and if the material is photochemically active, asymmetric photolysis should give rise to unequal decomposition of the enantiomers. For example, if left circular light is more strongly absorbed by (+) isomers, then the right circular light is preferentially absorbed at the same wavelength by the (—) isomers. Hence right circular incident light is more strongly absorbed by the (—) components of a racemate. If these molecules are decomposed by the light, then the (+) isomer should predominate in the product mixture.

Likewise, one might expect that under the dissymmetric influence of circularly polarized light, asymmetric photosynthesis might be achieved as well as asymmetric photodecompositions.

In order for a dissymmetric physical element, such as circularly polarized light, to modify a product ratio successfully, it is necessary that the

agent be essential to the reaction rather than play an inactive environmental role. Some of the early investigations, subsequent to Van't Hoff's suggestion to use circular light were doomed to failure, for although this physical agent is indeed dissymmetric, it is not in any way involved in many of the chemical reactions investigated, (For example, the experiments by Guye and Drouginine (1909)). A mechanistic requirement for observable asymmetric synthesis is that products must arise directly from the primary photochemical process, since the probability of asymmetry persisting through a chain reaction of any appreciable l e n g t h is vanishingly small.

The above conditions were not fulfilled in the experiments by Cotton (1896) with alkaline copper tartrate solutions. This m a t e r i a l exhibits strong circular dichroism in the red region of the spectrum. It also undergoes decomposition in sunlight. However, Byk (1904) demonstrated that the decomposition is caused by ultraviolet light (350-400 mu) and that alkaline solutions of copper tartrate are not acted on by red light as Cotton had thought. Furthermore, Mitchell (1930) could not detect any circular dichroism in the ultraviolet region. Thus upon irradiation with circular light of any wavelength, both enantiomers are affected to the same extent.

Quantitative Considerations

The magnitude of the expected activation is dependent upon the length of the photochemical influence and is always proportional to the relative difference of the extinction coefficients of the enantiomers. The latter parameter has been defined by Kuhn (1930) as the anisotropy factor:

$$g = \frac{\varepsilon^+ - \varepsilon^-}{\varepsilon}$$

where ε^+ and ε^- are the extinction coefficients of the (+) and (—) isomers, respectively, for light circularly polarized in a given sense. E is the extinction coefficient of the racemate. Kuhn shows that the anisotropy factor must also be equal to:

$$g = \frac{n_l - n_r}{n - 1}$$

where n_l and n_r are the different indices of refraction for left and right circularly polarized light respectively. Since it has been shown, Djerassi, (1960) that the rotatory power of an optically active medium is:

$$\phi = \frac{\pi}{\lambda}(n_l - n_r)$$

where ϕ is the rotation per centimeter for light of vacuum wavelength, it follows that:

$$g = \frac{\phi\ \lambda}{\pi\ (n - 1)}$$

Thus the anisotropy factor and subsequently the asymmetric efficiency may be evaluated either by rotation or absorption measurements. In some cases where optically pure enantiomers are not available, and consequently g is experimentally inaccessible, the asymmetric efficiency may be approximated from the anisotropy factor which is inversely proportional to f, the oscillator strength of the photochemically active absorption band (Kuhn, 1930). The oscillator strength is a constant times the integrated intensity of the band:

$$f = \frac{mc}{\pi e^2}\ \frac{1}{L}\ \int u\,d\nu$$

where c = velocity of light, e and m are the charge and mass of an electron, L = Loschmidt number (number of molecules per unit volume) and $\int u\,d\nu$ represents the area of the absorption band, u being defined by:

$$I/I_0 = e^{-ud}$$

It is unfortunate that Kuhn, in all his papers, used the symbol ε for absorption coefficient instead of molecular extinction coefficient; thus the ε has been changed to u in this paper to avoid discrepancy with the convention in common use in absorption spectroscopy as defined in the International Critical Tables.

To a first approximation

$$g = 10^{-5}/f$$

Note that only weak absorption bands can exhibit strong circular dichroism or have a high anisotropy factor. Hence, it is necessary to use materials which, in addition to possessing a high rotatory power in their optically pure state, exhibit photochemically and optically active absorption bands of low extinction in order to attain an observable activation of the racemate photochemically.

The magnitude of rotation expected after irradiation with circular light has been related to the anisotropy factor by Kuhn and Knopf (1930). The basic assumption involved in this relationship is the Einstein equivalent law: one and only one absorbing molecule will decompose after absorbing a light quantum hν. This assumption is of utmost importance in the quantitative evaluation of the rate of dissymmetric transformation, and thus the expected activation. If the mechanism of a photodecomposition involves a chain reaction, it is apparent that the asymmetric efficiency will be less than expected. The rates of change of concentration of (+) and (—) isomers due to asymmetric photolysis are:

$$-\frac{dc^+}{dt^+} = I_r^+ \frac{1}{h\nu v} \quad \& \quad \frac{dc^-}{dt^+} = I_r^- \frac{1}{h\nu v}$$

where I_r^+ & I_r^- are the relative amounts of energy absorbed from right circular light (I_r) per unit time by the (+) and (—) isomers, respectively, and v is the total volume of the sample. But since

$$I_r^+ = I_r \frac{\varepsilon_r^+ C^+}{\varepsilon_r^+ C^+ + \varepsilon_r^- C^-}$$

and

$$I_r^- = I_r \frac{\varepsilon_r^- C^-}{\varepsilon_r^+ C^+ + \varepsilon_r^- C^-}$$

where ε_r^+ is the extinction coefficient of (+) isomers for right circular light:

$$-\frac{dc^+}{dt^+} = I_r \frac{\varepsilon_r^+ C^+}{\varepsilon_r^+ C^+ + \varepsilon_r^+ C^+} \frac{1}{h\nu v}$$

and

$$-\frac{dc^-}{dt^+} = I_r \frac{\varepsilon_r^- C^-}{\varepsilon_r^+ C^+ + \varepsilon_r^+ C^+} \frac{1}{h\nu v}$$

Upon integrating the above equations, one obtains an expression for C^+ and C^-. It can then be shown that:

$$C^+ - C^- = 2\, C_0\, g/2\, (1-\delta)\, \ln \frac{1}{1-\delta}$$

where δ is the fractional part of material decomposed and $2\, C_0$ is the initial concentration of the racemate. A more useful form of this equation relates the degree of photolysis with the optical rotation.

$$\alpha = G\, g/2\, (1-\delta)\, \ln \frac{1}{1-\delta}$$

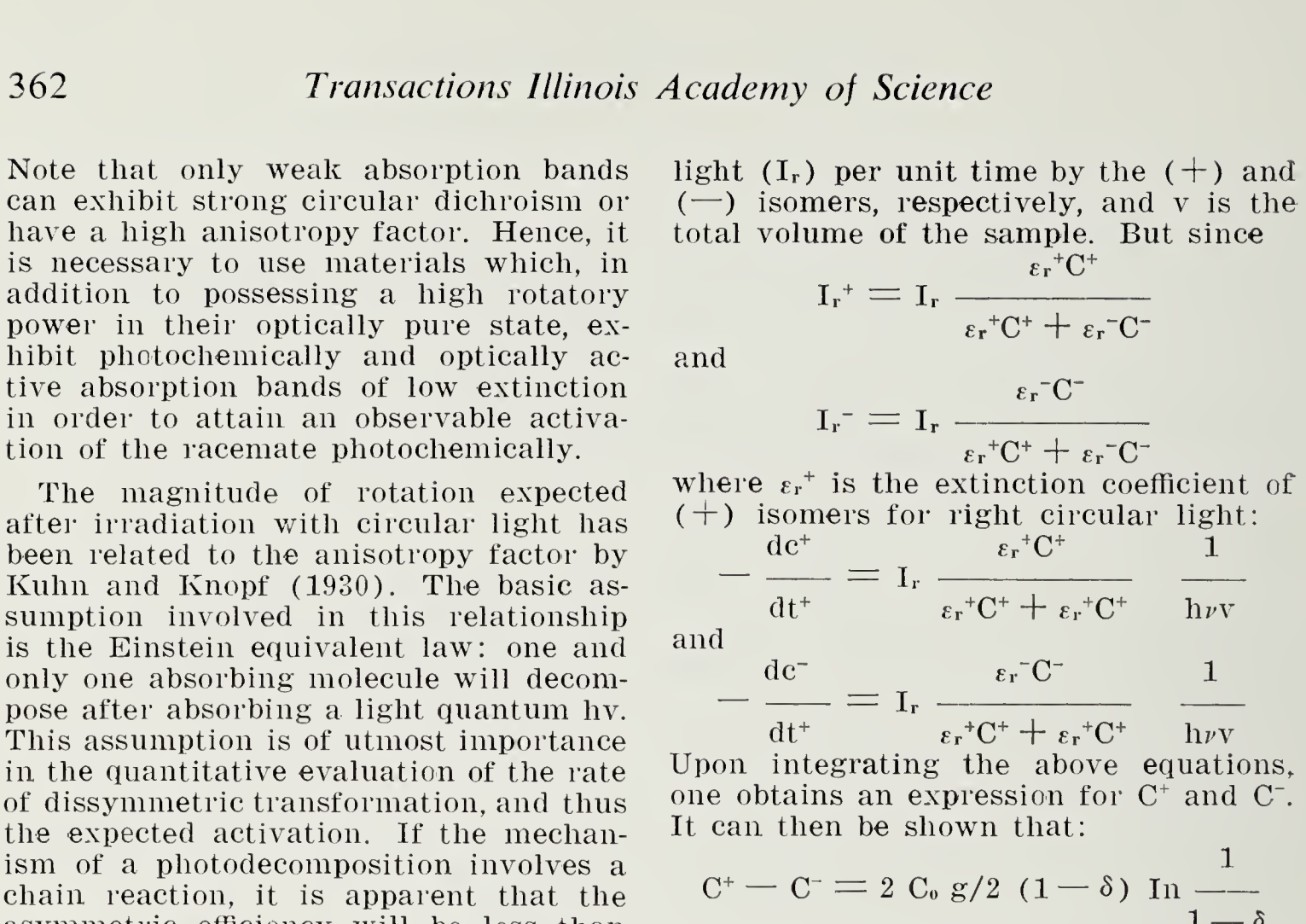

FIGURE 1.—A plot of optical activation as a function of the degree of dissymmetric photodecomposition.

where G represents the rotation that would be observed in an equivalent optically pure sample. Note that α is maximum when $\delta = 0.632$ (63.2% decomposition). This can easily be shown by setting $\frac{d\alpha}{d\delta} = 0$ and solving for δ.

Figure 1 shows how the activation $(C^+ - C^-)$ depends on the degree of decomposition (δ). Note that maximum activation corresponds to .368 $C_0 g = (1 - \delta)\ C_0 g$; $((1 - \delta)\ C_0$ is the quantity of non-reacted material). It is apparent that only low yields are possible by this method. At the onset of the experiment (in the region of positive slope) one enantiomer is decomposed more rapidly than the other due to its higher extinction coefficient for circular polarized light of a given sign. When a concentration difference between the enantiomers exists the solution is optically active. However, this relative difference in photolysis rate can only persist for a limited time (until 63.2% of the material is decomposed). Eventually the difference in concentration of the enantiomers is such that both are decomposed at the same rate. Ultimately all of the material is decomposed and the activation $(C^+ - C^-)$ is diminished to zero. Hence it is only possible to attain a partial dissymmetric transformation of the racemate by an asymmetric photolysis. For typical systems where $g = 10^{-2}$ the maximum percentage of activation is

$$\frac{1C^+ - C^-1}{C_0} \times 100 = 0.4\%$$

Figure 2 illustrates experimental data obtained by Mitchell (1930) on the decomposition of humulene nitrosite with right and left circularly polarized light. It should be kept in mind that δ will not be linear with time and hence, a plot of α (which is proportional to $C^+ - C^-$) vs δ will not be expected to have the same shape as α vs time.

At best this method of producing optically active materials is very inefficient and is primarily of academic significance.

Absolute Asymmetric Synthesis Via Asymmetric Photolysis

After several investigators (Cotton (1896), Freundler (1907), Guye and Drouginine (1909), Henle and Haakh (1908) and Bredig (1923)) failed to verify Van't Hoff's prediction, Zocher (1925) made a very encouraging and significant observation. He noted that sheets of silver halides became temporarily optically active after they had been irradiated with circularly polarized light. No activity was observed if the same substance was irradiated with plane polarized or non-polarized light. Although this experiment cannot be considered a true absolute asymmetric synthesis, since silver halides have neither an inherent "molecular" asymmetry nor lattice dissymmetry, it did indicate the need of utilizing a photochemical reaction in which the light plays an active and essential role. In this unique experiment, the silver halide lattice was rendered temporarily dissymmetric by the nature of the photoactivation.

Kuhn and Braun (1929) were apparently the first to succeed in an absolute asymmetric photolysis. In 1929 they investigated the photodecomposition of the ethyl ester of α-bromopropionic acid. This material has an ultraviolet absorption band and a corresponding Cotton effect around 245 mu. When a racemic mixture of the ester (5% in ethanol) was sealed in a quartz cuvette and exposed to circular polarized light (Mg line - 280 mu), an activation was achieved. The extent of decomposition was determined by simultaneous exposure of a control sample to non-polarized light from the same source. The largest activity observed was around 0.05° after 50% decomposition.

Later Kuhn and Knoph (1929, 1930) effected a successful asymmetric photolysis of the dimethylamide of α-azido-propionic acid,

$CH_3CHN_3CON(CH_3)_2$, with circularly polarized light of wavelength 280-310 mu. The azido compound has a photochemically active absorption band in the ultraviolet spectrum at 290 mu. The extent of photodecomposition may be conveniently determined by measuring the amount of N_2 evolved. In this experiment $G = 340°$, $g = 0.024$, $\delta = 0.40$ (40%). On the theoretical grounds previously cited, the expected activation was: $\alpha = 1.25°$. The observed rotations were $\alpha_D = -1.04$ and $\alpha_D = +0.78$ after irradiation with left and right circular light respectively. Since the light was not exactly, but only approximately circularly polarized and furthermore, since the Einstein equivalent law (which was assumed) represents an idealized system, the theoretical treatment previously described and the understanding of the activation process may be considered as being confirmed.

Similar experiments utilizing the same technique have been carried out by Mitchell (1930) with humulene nitrosite and more recently by Berson and Brown (1955) with 4-(2′-nitrophenyl) -1,4-dihydropyridine. Tsuchida, Nakamura and Kobayashi (1935) have reported success with an asymmetric photolysis of K_3 [Co (oxylate)$_3$]. Apparently this has been the only successful attempt to carry out such an experiment with a metallic complex. K_3 [Co (oxylate)$_3$] possesses an absorption band at 605 mu with an anisotropy factor in the order of 10^{-2} in magnitude. Asymmetric photolysis was carried out with D-line radiation. Rotation measurements were made at 499 mu to avoid any accidental photolysis during the measuring periods. Maximum activation ($\alpha = .16°$) was observed after 8.5 hours of illumination.

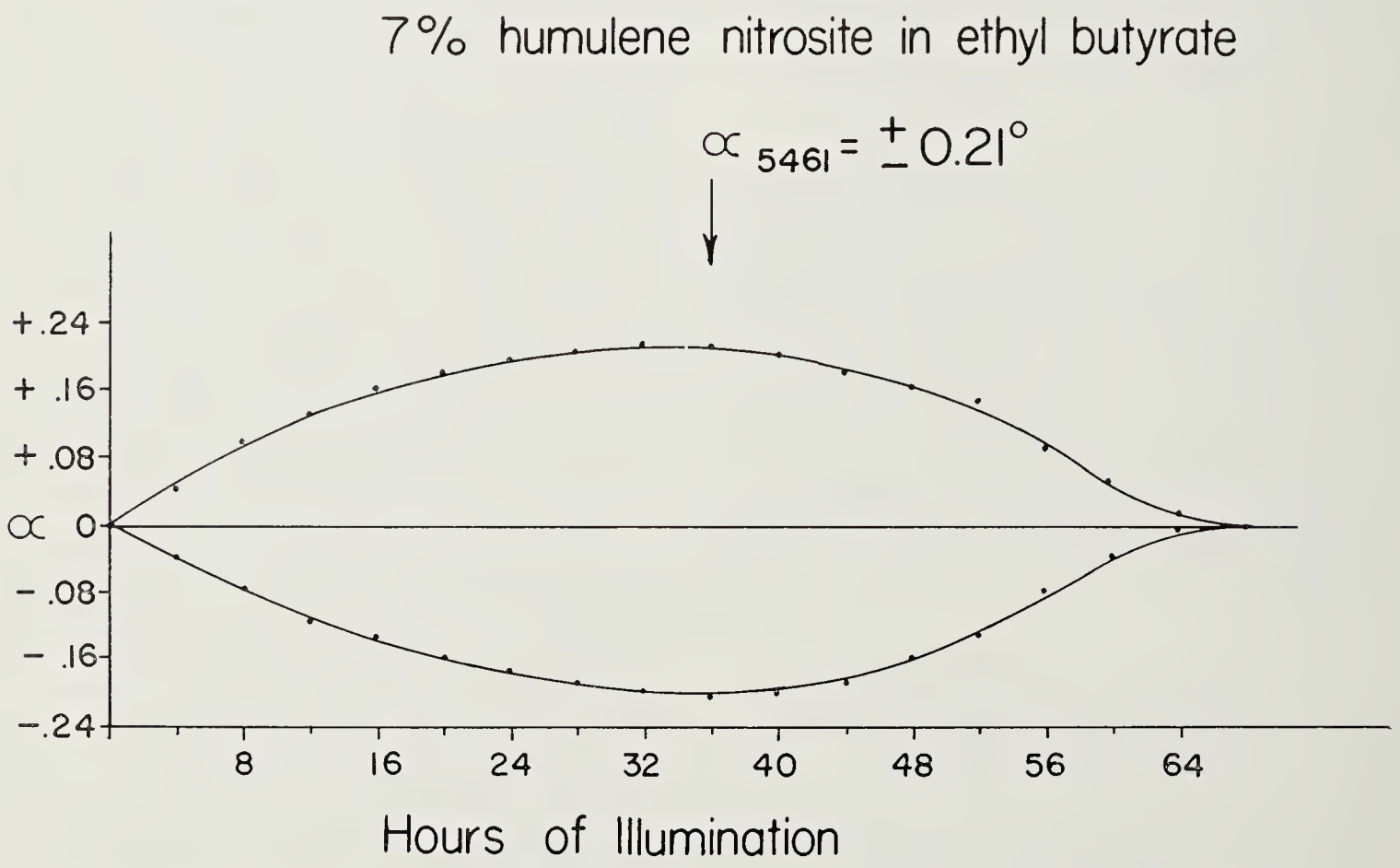

FIGURE 2.—Optical rotation of humulene nitrosite irradiated with right and left circularly polarized light (Mitchell (1930)).

Absolute Asymmetric Synthesis Via Asymmetric Photosynthesis

Whereas the particular experiments cited invoke asymmetric destruction, cases of absolute asymmetric synthesis under the influence of circularly polarized light have also been reported, although the observed rotations of the products were very small (less than 0.2°). Davis and Heggie (1935) reported the asymmetric photochemical addition of bromine to 2,4,6-trinitrostilbene under the influence of circularly polarized light of wave length 360-450 mu. Trinitrostilbene would seem to be particularly suited to the purpose of asymmetric synthesis since dark reaction is negligible, it possesses an absorption band in the visible spectrum, and since the phenyl and trinitrophenyl group differ markedly from one another in their electrical character, the ethylenic linkage has a polar character. They were successful in producing optically active trinitrostilbene dibromide by the photochemical addition of reagents under the influence of circularly polarized light in carbon tetrachloride and in nitrobenzene. Betti and Lucchi (1938) carried out a similar experiment in which the asymmetric photochemical addition of chlorine to an ethlylenic linkage was attained with both gaseous reactants and products. Karagunis and Drikos (1933, 1934) synthesized optically active triarylmethylchlorides from triarylmethyl radicals and chlorine. They have suggested that the three valencies of the carbon atom of the radical do not lie on a plane. Thus the radical itself, although racemic, is asymmetric. They propose that circularly polarized light activates one of the enantiomers so that by the addition of chlorine, optical activity appears.

It is of interest to note that Souty (1934) has reported the rate of mutarotation of certain carbohydrates is different in right and in left circularly polarized light of wave length 546 mu, but is the same in right and in left circularly polarized light of wavelength 589 mu.

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Manuscript received November 15, 1967.

EXPERIMENTAL RELEASES OF PHEASANTS IN WABASH COUNTY, ILLINOIS

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ABSTRACT. — Four strains of the ring-necked pheasant (*Phasianus colchicus*) and one of the green pheasant (*P. versicolor*), totaling 1,155 birds, were experimentally released on an area near Bellmont in Wabash County, March 2-April 12, 1963 and March 18-19, 1964. Bellmont is located about 85 miles south of the established pheasant range in Illinois. Counts of territorial cocks during May indicated that the breeding population of cocks totaled 21, 31, 21, 10, and 5, respectively, in the 5 years 1963 through 1967. Sex ratios (cocks per hen) among birds observed during May through August were 0.47 and 0.65 in 1963 and 1964, respectively, but increased to 2.73 in 1965. The number of broods located on the area was 52 in 1963, 52 in 1964, and 11 in 1965. Thus, the population rapidly declined after the releases were discontinued and was practically nonexistent by 1967. Evidence indicated factors that caused the population to fail were concerned more with survival of the birds than with reproduction. Of an estimated 2,091 pheasants released and hatched on the area in 1963 and 1964, only two per cent survived to May 1965. Sex ratios among birds observed or captured by nightlighting during October and early November indicated that hens experienced higher rates of mortality than cocks. Mean weights of juvenile cocks and of juvenile hens captured at Bellmont were similar to those characteristic of juvenile pheasants in the established range in east-central Illinois.

The exotic ring-necked pheasant (*Phasianus colchicus*) has been established throughout the northeastern third of Illinois for more than 30 years (Greeley et al. 1962:13-16). A census conducted in April 1963 indicated that this pattern of distribution has not changed materially in recent years (Labisky and Anderson 1965:-132). Numerous attempts to establish pheasants in the southern half of the state, by releases of birds, have failed (Leopold 1931:106; Ellis and Anderson 1963:238). Because pheasants are important game birds and are able to tolerate intensive land-use practices characteristic of modern farming, repeated attempts have been made to extend the range of these birds in Illinois.

This paper discusses the fate of a population of pheasants created by experimental releases of four strains of *P. colchicus* and one of *P. versicolor* in 1963 and 1964 on an area near Bellmont in Wabash County. The releases were made to test the ability of crosses among various species, subspecies, and strains of *Phasianus* to produce self-maintaining populations south of the established range. It was theorized that, through the mechanics of population genetics, a pheasant that would survive and reproduce in agricultural areas unoccupied by pheasants might develop if several species, subspecies, and strains

were released simultaneously. Releases of pheasants in this manner would increase variation of genetic material in the resulting population. In theory, the greater the genetic variation within the population, the greater the chances for producing a bird that would survive and reproduce under the existing environmental conditions. The releases at Bellmont initiated the second *multiple-strain* — or *gene pool*—experiment in Illinois, the first one being on an area near Neoga, Cumberland County, where pheasants were released from 1960 through 1963 (Anderson 1964*a*, 1964*b*, and unpublished data).

Release Area

The Bellmont Area occupies about 15 square miles of level to moderately rolling farmland in southwestern Wabash County, approximately 85 miles south of the contiguous pheasant range in Illinois. Soils are relatively young and fertile, generally dark in color, tile drained, and slightly acid to neutral (Smith and Smith 1937:19-26).

Ninety per cent of the release area is considered agricultural land. Corn, soybeans, and wheat collectively occupy about 75 per cent of the total land area annually; 10 per cent of the area is devoted to pasture and hay. Woodlots comprise the bulk of the nonagricultural land.

The climate in the vicinity of Bellmont is warmer and wetter than within the contiguous pheasant range in Illinois. Annual precipitation at Mt. Carmel, located 8 miles east of Bellmont, averages 43 inches; temperatures average 33°F during January and 79°F during July (Page 1949:115, 117, 283). As a comparison, Roberts, located in Ford County — the heart of Illinois' better pheasant range — receives an average of 33 inches of precipitation annually, and temperatures during January and July average 27°F and 76°F, respectively (Page 1949:115, 117, 260).

Methods

The following strains of pheasants were involved in the multiple-strain experiment at Bellmont:

Korean pheasant — Supposedly pure Korean ring-necked pheasants (*P. colchicus karpowi*, nomenclature by Delacour 1951:257), obtained from Korea by the Foreign Game Introduction Program sponsored by the U.S. Bureau of

Table 1.—Strains and Numbers of Pheasants Released at Bellmont, Illinois, in 1963 and 1964.

Strain	Year Hatched	Date Released	Number Released	
			Cocks	Hens
1963				
Wild-Illinois	Mixed[1]	March 2	14	0
	Mixed[1]	April 12	20	0
California	1962	March 22	0	54
	1962	April 3	0	300
Totals			34	354
1964				
Wild-Iowa	Mixed[1]	March 18	71	0
California	1963	March 19	0	500
Korean	1963	March 18	50	81
Green	1963	March 18	0	65
Totals			121	646

[1] Birds hatched the previous year and older birds.

Sport Fisheries and Wildlife, and propagated at Illinois state game farms.

California pheasant—Ring-necked pheasants (*P. colchicus*) originating from stock obtained from game farms located at Chico and Yountville, California (Ellis and Anderson 1963:226) and propagated at Illinois state game farms.

Wild-Illinois pheasant — Ring-necked pheasants (*P. colchicus*) captured from thriving wild populations in Ford and Champaign counties, east-central Illinois.

Wild-Iowa pheasant—Ring-necked pheasants (*P. colchicus*) captured from thriving wild populations in Union and Adair counties, southwestern Iowa.

Green pheasant—Supposedly pure northern green pheasants (*P. versicolor robustipes*, nomenclature by Delacour 1951:265), obtained from the island of Honshu, Japan, by the Foreign Game Introduction Program and propagated at Illinois state game farms.

Native pheasant — Birds hatched and reared on the release area, i.e., progeny of the introduced pheasants.

Pheasants released in 1963 were 34 wild-Illinois cocks and 354 California hens, resulting in a sex ratio of 0.10 cock per hen (Table 1). In 1964, birds of wild-Iowa, California, Korean, and green stock, totaling 121 cocks and 646 hens (0.19 cock per hen), were liberated.

The wild-Illinois and wild-Iowa birds had been captured by nightlighting (Labisky 1959). The wild-Iowa pheasants were held in large covered pens near Boone, Iowa, for about 3 months prior to their release at Bellmont. Wild-Illinois birds were retained in captivity for not more than 36 hours.

Wild-Illinois, wild-Iowa, and Korean birds were marked with numbered, plastic back tags (Labisky and Mann 1962) and aluminum, butt-end leg bands. Back tags were color coded according to origin of the birds and year of release. California and green pheasants, all hens, were marked only with leg bands. Because of their dark plumage, green hens were readily distinguishable from hens of other strains. All of the introduced pheasants were released onto the Bellmont Area directly from transporting crates. Most of the birds were in good physical condition.

Counts or estimates were periodically made of various cohorts of the population. During May, the number of territorial cocks on the area was determined by a combination of audio-censuses (Graham 1940:313) and visual observation. The sex ratio among adult pheasants observed from May through August was used as an index of the relative abundance of hens present during the nesting season.

The number of broods produced was determined by locating individual broods by direct observation and by keeping records of broods observed and reported by farmers. During June through August, all roads on the study area were driven during early morning and late afternoon to locate and observe broods and adults. The average number of chicks per brood was determined from observations for which the counts of chicks were considered to be complete. Chicks in broods were aged to the nearest week according to body size and development of plumage. Most of the pheasant nests examined were found and reported by farmers.

In 1963 and 1964, an index of the abundance of pheasants during the prehunt period (early November) was obtained by counting the number of birds flushed per truck-hour of nightlighting. Roosting cover (hayfields and wheat stubble) was systematically searched at night using a truck with a cluster of floodlights mounted on its top. Pheasants can be quickly found and, with the aid of a hooped net, captured with this technique (Labisky 1959:10).

In 1964, the prehunt population was estimated using the Lincoln Index; pheasants were captured by nightlighting during October and early November, marked with back tags and leg bands, and released in the fields where they had been captured. Subsequently, local farmers and sportsmen were asked to keep records of the numbers of marked and unmarked pheasants they saw while hunting on the area. This information provided three known values, from which the prehunt population was calculated using the simple ratio formula. The sex and age composition of the prehunt populations was determined from pheasants observed and captured during nightlighting. Captured birds were aged as juveniles (less than 1 year old) or adults by bursal examination.

Results

Counts of territorial cocks during May suggested that the breeding population of cocks increased from 1963

TABLE 2.—Status of the Pheasant Population at Bellmont, Illinois, 1963 through 1967.

	Month	1963	1964	1965	1966	1967
Breeding Cocks	May	21	31	21	10	5
Observed Sex Ratio: Cocks per Hen	May-Aug.	0.47	0.65	2.73	2.00	.....[1]
Chicks Produced	June-Aug.	468	468	99	.....[1]	.....[1]
Prehunt Population	October	556	239	.....[1]	.....[1]	.....[1]

[1] No data available.

to 1964, then steadily decreased through 1967 (TABLE 2). Sex ratios among birds observed during May through August indicated that hens were more abundant than cocks during the years pheasants were released, 1963 and 1964, but became relatively more scarce during the following years (TABLE 2). As the number of cocks per hen among pheasants released was only 0.10 in 1963 and 0.19 in 1964, this finding was not surprising. When the observed sex ratios were evaluated in conjunction with the counts of territorial cocks, it was apparent that the number of hens on the area each spring decreased at a drastic rate after 1964 (TABLE 2). Observations of pheasants during the summer months indicated that wild-Illinois and wild-Iowa cocks were more successful than Korean cocks in surviving at Bellmont (TABLE 3). Similarly, California hens apparently enjoyed better survival than did hens of the Korean and green strains (TABLE 3). Hens of the latter two strains, released in 1964, were not observed during the June, July, or August following their release. California

TABLE 3.—Number of Adult Pheasants Observed, Including Repeat Observations of Individual Birds, during May through August, 1963 through 1966, Bellmont, Illinois. Percent of the number released the preceding March or April is given in parentheses.

Strain of Pheasant	Cocks				Hens			
	1963	1964	1965	1966	1963	1964	1965	1966
Wild-Illinois	20(59)	0	0	0				
California					43(14)	43(9+)	2	0
California or Native[1]						54		
Wild-Iowa	.....	41(58)	5	1				
Korean	.....	10(20)	0	0	.....	2(2)	0	0
Green					.....	7(11)	0	0
Native	.....	24	25	5	.....	9	9	3
Totals	20	75	30	6	43	115	11	3

[1] In 1964, it was frequently impossible to distinguish between native hens and California hens.

hens, liberated in 1963 and 1964, were observed on the area through August of both years.

Five pheasant nests were found at Bellmont in 1963, 13 in 1964, and one in 1965. Six of the 19 clutches had been incubated and seven had not; the status of the other six clutches was undetermined. Three of the clutches had hatched. All five nests found in 1963 had been established by hens from California stock, the only strain of hen pheasant present that year. In 1964, the hens that established six nests were identified as California or native birds (evidence was insufficient to distinguish between hens of these two strains). Origins of the hens that established the other seven nests found in 1964, plus the one located in 1965, was undetermined. Korean and green hens were never associated with any of the nests found on the area.

The six incubated clutches examined contained an average of 11.7±2.2 eggs. An average of 9.0±2.0 hatched eggs was found in the three successful clutches. These data compare favorably with similar information reported for several areas within the established pheasant range in North America (Stokes 1954:27, 33).

The number of broods located at Bellmont totaled 52 in 1963, 52 in 1964, and 11 in 1965. In 1963, the only hens present to produce broods were those from California stock. In 1964, the origins of the hens that accompanied, and presumably produced, 16 broods were determined; 15 were California and one was a Korean. Twenty-seven of the remaining 36 hens were known to be either California or native birds (progeny of California hens and wild-Illinois cocks), but their exact identity was not determined. The origin of only one brood-producing hen was determined in 1965; she was a native.

Although green hens were never observed with any of the broods located on the area, two territorial cocks observed during April 1965 appeared to be green-ringneck hybrids. These cocks had reduced neckrings (completely absent on ventral) and prominent green coloration on the lower portion of the neck; their calls were more high pitched than those of typical ringneck cocks.

The number of chicks produced (TABLE 2) was estimated by multiplying the number of broods located by 9.0 hatched eggs per successful clutch. Although the average of 9.0 hatched eggs was based on only three successful clutches, all found in 1964, this average is similar to those obtained on a release area near Neoga in Cumberland County in 1960 and 1961 (Anderson 1964*a*:259) and to those within the range occupied by self-maintaining populations of pheasants (Stokes 1954:33).

Ninety-two per cent (22 of 24), 56 per cent (22 of 39), and 25 per cent (1 of 4) of all adult hens observed between June 15 and August 31 in 1963, 1964, and 1965, respectively, were accompanied by broods. Considering the small sample sizes, these percentages are in the general range of those reported for established populations in Pennsylvania (Randall 1940:316), Ohio (Leedy and Hicks 1945:66), and Michigan (Shick 1952:22). The number of chicks per adult hen observed at Bellmont between June 15 and August 31 was 7.4 (177 chicks and 24 hens)

in 1963, 3.0 (118 chicks and 39 hens) in 1964, and 4.0 (16 chicks and 4 hens) in 1965. These findings suggest that surviving hens on the Bellmont Area were reasonably successful in producing chicks in 1963 and 1964. The data obtained in 1965 were too few to evaluate.

Averages of 7.5±0.3 chicks (average age 6.7 weeks), 5.9±0.2 chicks (average age 5.9 weeks), and 3.7±1.2 chicks (average age 6.0 weeks) were calculated for 21, 14, and 3 broods observed, respectively, in 1963, 1964, and 1965. One brood, containing six chicks 4 weeks old, was observed in 1966. On an area near Sibley in Ford County, near the center of abundance of pheasants in Illinois, averages of 5.2, 4.7, and 5.1 chicks were calculated for broods observed in 1963, 1964, and 1965, respectively (S. L. Etter, Illinois Natural History Survey, unpublished data). Therefore, it appears that, in general, the number of chicks produced per successful clutch and subsequent survival of chicks through at least their 6th week of life was not suppressed at Bellmont.

In 1963 and 1964, pheasants were observed and captured during the prehunt period (TABLE 4). The average numbers of birds flushed per truck-hour during nightlighting indicated that the abundance of pheasants on the area declined 43 per cent from early November 1963 to about the same period in 1964.

In 1964, the prehunt population was estimated to be 239 pheasants (46 birds marked during prehunt period × 83 birds observed by hunters ÷ 16 of the observed birds were marked = 239 pheasants). The sex ratio among all pheasants flushed and age ratios among cocks and hens captured (TABLE 4) indicated the 239 birds included 138 cocks (122 juveniles and 16 adults) and 101 hens (73 juveniles and 28 adults). This estimate of the sex and age composition of the prehunt population suggested that only 42 per cent (195

TABLE 4.—Population Data Obtained by Nightlighting Pheasants at Bellmont, Illinois, November 7-8, 1963 and October 19-November 5, 1964.

Type of Information	1963		1964	
Number of Pheasants Flushed				
Total	55		85	
Per Truck-hour	6.9		3.9	
Number of Pheasants Captured				
Total	38		51	
Per Truck-hour	4.8		2.3	
	Cocks	Hens	Cocks	Hens
Sex Composition of Pheasants Flushed	35	20	49	36
Age Composition of Pheasants Captured				
Juveniles	25	4	23	18
Adults	2	7	3	7

birds) of the 468 chicks estimated to have hatched on the area in 1964 survived to the following October. The estimate of 28 adult hens in the prehunt population indicated that less than 4 per cent of the 646 hens released in March 1964, survived to the following October. This exceedingly low survival of hens was unquestionably detrimental to the population.

The predominance of cocks among pheasants flushed by nightlighting (TABLE 4) was indicative of differential rates of mortality between the sexes during summer and early fall. Ratios of 1.75 and 1.36 cocks per hen occurred, respectively, among birds flushed in 1963 and 1964. The sex composition of juveniles captured was 6.25 cocks per hen in 1963 and 1.28 cocks per hen in 1964.

Age ratios, expressed as juveniles of both sexes per adult hen, were 4.1 and 5.9, respectively, among pheasants captured during the prehunt periods in 1963 and 1964 (TABLE 4). Such ratios are indicative of moderately high productivity.

Mean weights of juvenile hens captured in 1964 and of juvenile cocks captured in 1963 and in 1964 (TABLE 5) were similar to those characteristic of juvenile pheasants in established populations in Ford County (R. F. Labisky, Illinois Natural History Survey, unpublished data). Although the mean weight of juvenile hens captured in 1963 appeared to be subnormal, the small sample size seriously impaired validity of the data. No aberrancies were noted in the mean weights of adult hens, nearly all of which were of California stock (TABLE 5).

DISCUSSION

The findings suggest that pheasants were relatively abundant on the Bellmont Area in 1963 and 1964, the years of the releases, and relatively scarce thereafter (TABLE 2). Counts of territorial cocks indicated the population was, for practical purposes, nonexistent by 1967. Thus, the pheasants involved in the multiple-strain releases at Bellmont did not

TABLE 5.—Mean Weights, In Grams, of Pheasants Captured by Nightlighting during November 7-8, 1963 and October 19-November 5, 1964, Bellmont, Illinois. Sample sizes are in parentheses.

Sex and age	1963	1964	Both Years
Cocks			
Juveniles[1]	1,243±16 (25)	1,246±15 (18)	1,244±13 (43)
Adults	1,319±43 (2)	1,385±4 (3)	1,358±22 (5)
Hens			
Juveniles[1]	800±44 (4)	879±13 (9)	855±18 (13)
Adults	909±20 (7)[2]	929±18 (6)[3]	918±13 (13)

[1] All juveniles weighed were at least 15 weeks old as determined by progression of molt of the primary flight feathers.
[2] All were California hens.
[3] Five were California hens, and one was a native that weighed 935 g.

develop a bird during the relatively short period, 1963 to 1967, which was adapted to the environmental conditions that prevail in Wabash County.

Experimental releases, involving birds of California stock only, on this same area in 1957 and on another area, located near Neoga in Cumberland County, in 1956 had previously failed to establish pheasants in the southern half of the state (Ellis and Anderson 1963:238). The populations created by these releases declined to a relatively few birds within 3 years. However, releases at Neoga during the winters of 1959-60 through 1962-63, involving several different strains of pheasants, resulted in a small population that persisted at least to 1967 (W. L. Anderson, unpublished data). Thus, the multiple-strain experiment at Bellmont experienced a fate similar to those of the earlier experiments involving California birds only. In other words, the simultaneous release of five strains of pheasants on this area did not materially delay expiration of the resulting population.

The reasons that the multiple-strain experiment at Bellmont was less "successful" than the one at Neoga are obscure. However, the releases at Neoga were made during 4 consecutive years and involved 2,662 pheasants, as compared with 1,155 birds released during 2 consecutive years at Bellmont. It is possible these differences contributed to the differences noted in the fates of the two multiple-strain experiments. It is also possible that the factors preventing establishment of pheasants in southern Illinois are more severe at Bellmont, which is located 85 miles south of the established range, than at Neoga, which is only 20 miles south of self-maintaining populations. Finally, the releases at Neoga coincided with a period of increase in pheasant abundance throughout much of the established range of this species in Illinois, particularly in the east-central counties, whereas releases at Bellmont coincided with years of decline throughout much of the occupied range in the state.

Earlier studies (Ellis and Anderson 1963:238; Anderson 1964*a*: 263) indicated that the factors deterring the southward extension of self-maintaining populations of pheasants in Illinois are concerned more with survival of the birds than with reproduction. Findings of the present study tend to support this conclusion. Indices of reproductive success indicated that hens surviving at Bellmont were reasonably successful in hatching and rearing chicks to 6 weeks of age in 1963 and 1964.

The catastrophic rate of mortality that apparently occurred among pheasants at Bellmont was illustrated by the rapid deterioration of the population in 1965, the year after releases were discontinued (TABLE 2). Although 21 territorial cocks were located in 1965, the observed sex ratio (2.73 cocks per hen) indicated the number of hens was drastically reduced that year; it was believed the actual sex ratio was no less than one cock per hen in 1965. If this assumption is reasonably correct, only about two per cent of the 2,091 pheasants (1,155 birds released and 936 hatched) on the area in 1963 and 1964 survived to May 1965.

Sex ratios among pheasants flushed or captured by nightlighting during

the prehunt periods of 1963 and 1964 (TABLE 4) suggested that hens experienced higher rates of mortality than cocks. With the possible exception of juvenile hens weighed in 1963. no peculiarities were noted among mean weights of hens captured on the area (TABLE 5). However, body weight is by no means an absolute indicator of physical condition. Detailed physiological studies of pheasants subsisting on areas in southern Illinois could be highly rewarding in disclosing factors preventing the southward extension of pheasants in the state.

ACKNOWLEDGMENTS

Acknowledgments are made to the following personnel, past or present, of the Illinois Natural History Survey: Ronald F. Labisky and Wm. R. Edwards for technical supervision; Glen C. Sanderson and Helen C. Schultz for editorial advice; and Steven L. Wunderle for assistance in collecting data. Robert W. Touchberry, University of Illinois, offered advice on genetic aspects of the study.

This research is a contribution from Illinois Federal Aid Project No. W-66-R, the Illinois Department of Conservation, the U.S. Bureau of Sport Fisheries and Wildlife, and the Illinois Natural History Survey, cooperating.

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Manuscript received December 12, 1967.

FACTORS AFFECTING FOOD INTAKE BY THE SHORTNOSE GAR

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ABSTRACT. — Two experimental designs supplemented by field observations were utilized to investigate factors affecting the food intake by the shortnose gar and the effectiveness of this gar as a predator. Variations in susceptibility to capture on the part of the forage affected the gar's ability to utilize different organisms. The shortnose gar does not appear to be any more effective as a predator than does the largemouth bass and the channel catfish. During a part of the year invertebrate forms were a major item in the gar's diet. It was suggested that the "beak" of the gar is an adaptation for capturing benthic organisms. Rainfall caused a major increase in the utilization of terrestrial organisms.

On the basis of earlier studies (Lewis et al., 1961; Lewis and Helms, 1964; and Anthony, 1964) Lewis (1967) has pointed out that stomach analyses of fishes from established populations do not reveal the potential of a predator to utilize forms that are particularly susceptible to capture. In a given body of water such forms are rather rapidly eliminated or drastically reduced in number (Bremer, 1965). Thus, subsequently they do not appear as food items by stomach analysis. In the studies mentioned above, variation in susceptibility to capture between various organisms of forage size is demonstrated to exist. Lewis and Helms (1964) concluded that susceptibility to capture is a characteristic of the forage species and that the ability of different predator fishes to capture a given forage organism is similar. In the present investigations it was postulated that this conclusion might not hold for the gar pikes (*Lepisosteus*), which, according to the literature (TABLE 1), appear to be highly piscivorous, and which are phylogenetically separated and morphologically different from the largemouth bass (*Micropterus salmoides*), smallmouth bass (*M. dolomieui*), and channel catfish (*Ictalurus punctatus*), which were considered in the earlier studies.

Using the experimental design employed in the earlier works mentioned above and by collections of fish from the wild, an investigation was made of the ability of the shortnose gar (*L. platostomus*) to utilize various forage organisms, contributions of different food organisms to the seasonal diet of this gar in the wild, effects of rainfall on food intake, and effects of the presence of highly vulnerable forage on food intake.

PROCEDURES

Individual shortnose gar ranging in weight from 0.8 to 1.1 pounds were held

TABLE 1.—Stomach Contents of the Gar Pikes as Reported by Other Authors.

Reference	Species	Months of sample	Number of specimens	Percent stomachs full	Food items—percent					
					Fishes		Insects		Other	
					Occ.	Vol.	Occ.	Vol.	Occ.	Vol.
1	Alligator	.....	21	1	100	100	0	0	0	0
	Spotted	.....	28	28	100	.....	0	0	45	.....
	Longnose	.....	297	38	83	.....	14	.....	5	.....
2	Shortnose	Feb-June	2,410	8	76	.....	.....	.....	23	.....
	Shortnose	July-Aug	217	34	100	100	0	0	0	0
	Longnose	Jan-May	155	20	99	.....	1	.....	0	0
3	Spotted	Feb-June	448	23	70	76	19	8	38	16
4	Longnose	June-Aug	204	66	100	99	5	0	2	1
5	(not stated)	.....	89	44	94	.....	0	0	3	.....

References: (1) Bonham, K. (1940); (2) Holloway, A. D. (1954); (3) Hunt, B. P. (1952); (4) Lagler, K. F. and F. V. Hubbs (1940); (5) Scott, W. (1938).

in six stock watering tanks. The tanks were four feet in diameter, two feet deep, and were filled to the 16-inch level. The tanks were located indoors and were illuminated 12 hours each day. Two lots of gar were used; one in 1965 and another in 1966. It was necessary to hold each lot approximately a month in the presence of food before the fish commenced feeding. The first lot was held at 70 to 73°F., and the second lot at 83°F. In any one trial two forage species were offered. In the majority of trials the complement was maintained at five organisms of each species. In two trials ten individuals of each species were used since all of the tadpoles were eaten when only five were offered. In all cases the complement was replenished once each day. The duration of the trials varied as is indicated in TABLE 2. Utilization of the forage was measured in terms of number of forage organisms eaten per gar per day.

In evaluation of forage utilization in the pond habitat, a group of identical 0.1-acre drainable ponds were utilized. The ponds could be refilled by gravity from a reservoir. They were free of vegetation and had a shoreline slope of one to one with a depth of five feet at the drain and four feet at the upper end. The water turbidity ranged from 20 to 40 p.p.m. silicon dioxide and was due primarily to silt.

Each of three ponds was stocked with 500 of each species of the forage being investigated. In some cases this was one species, while in other cases two or three species were stocked. Two of the three ponds were then stocked with six gar, the size of which ranged from 0.5 to 1.5 pounds. The ponds were permitted to remain undisturbed for ten days. At the end of this time the gar were removed by seining, the ponds were drained and the remaining forage organisms counted. The difference in numbers of forage organisms between the pond without gar and those with gar was considered to be the forage utilized by the gar. To verify forage utilization, stomach analyses were made on half of the gar at the time they were removed.

Investigations of the feeding habits of the gar in the wild were carried out in the Big Muddy River, a large, turbid tributary of the Mississippi. Sampling was done two to three miles above the confluence of the river with the Mississippi. Fish were collected by use of electro-fishing equipment mounted on a boat. When possible, sampling was done once each week and an attempt was made to obtain 20 fish per trip. However,

sampling conditions did not always permit achieving these goals. Particular emphasis was placed upon determination of seasonal changes in food, the utilization of terrestrial vs. aquatic food, the utilization of invertebrate life vs. fishes, and the effect of rainfall upon stomach contents.

Results

Under conditions of confinement in the tanks there was significant utilization of all species of forage offered (Table 2). However, there occurred a greater utilization of tadpoles than of other forms. On the assumption that confinement rendered all of the forms more vulnerable, the greater utilization of tadpoles could indicate a preference for this organism. In the pond trials (Table 3) it is evident that vulnerability played a role similar to that observed in the earlier studies with bass and channel catfish. The utilization of less vulnerable forms such as the bluegill (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*) was less than that which occurred in the tanks. Utilization of the forms previously considered to be vulnerable was again high. Thus the utilization of bullfrog tadpoles (*Rana catesbeiana*), crayfish (*Procambarus blandingi*), and black bullhead (*Ictalurus melas*) fingerlings was notably high.

Table 2.—Utilization of Forage Organisms by Shortnose Gar in Tanks.

Forage species	No. organisms offered each day	Gar days	No. forage eaten/gar/day
Golden shiner	5	12	0.62
Bullfrog tadpole[1]	5		0.12
Pond crayfish	5	46	1.81
Fathead minnow	5		1.14
Fathead minnow	5	65	3.19
Bullfrog tadpole	5		2.00
Fathead minnow	5	25	2.52
Bullfrog tadpole	5		2.85
Fathead minnow	5	34	1.04
Bullfrog tadpole	5		5.00
Fathead minnow	5	20	2.70
Bullfrog tadpole	5		4.65
Bluegill	5	14	2.10
Bullfrog tadpole	5		5.00
Bluegill	10	64	1.45
Bullfrog tadpole	10		4.80
Black bullhead	5	7	3.10
European carp	5		3.11
Black bullhead	10	8	2.75
European carp	10		5.70

[1] Initial trial after confinement of shortnose gar in tanks may account for poor utilization.

TABLE 3.—Utilization of Forage Organisms by Shortnose Gar in 0.1-acre Ponds.

Forage species	Utilization-Organisms/gar/day				
	Trial 1	Trial 2	Trial 3	Trial 4	Mean
Crayfish	2.91	2.23	.....	.....	2.57
Crayfish	2.34	7.20	7.30	.....	5.61
Bullfrog tadpole	3.40	5.60	5.65	.....	4.85
Golden shiner	0.10	0.33	0.00	0.00	0.11
Crayfish	3.58	2.57	2.12	0.80	2.76
Bullfrog tadpole	2.74	2.04	2.18	0.58	1.88
Fathead minnow	0.30	0.28	0.33	0.22	0.28
Bullfrog tadpole	3.32	2.71	6.30	5.60	4.48
Fathead minnow	1.15	0.30	0.90	0.23	0.64
Bluegill	0.00	0.00	0.48	2.39	0.72
Fathead minnow	1.13	1.56	.....	.....	1.34
Black bullhead	2.96	5.79	.....	.....	4.37
Black bullhead	4.35	3.92	.....	.....	4.13
Bullfrog tadpole	3.91	6.62	.....	.....	5.26
Black bullhead	5.90	6.40	.....	.....	6.15
European carp	1.85	2.80	.....	.....	2.32

The stomach contents of 102 shortnosed gar taken from the wild revealed a greater utilization of insects than has been previously reported. A large percent of this material was terrestrial beetles. The benthic organisms consumed consisted almost entirely of naiads of one of the burrowing mayflies (*Hexagenia* sp.).

With the progression of the season there occurred a notable shift in the food utilized. During some periods terrestrial and nektonic insects were most heavily utilized, while during others benthic invertebrates or fishes were more important (TABLE 4). Rainfall affected both the composition of the stomach contents and the level of intake. The percent of stomachs containing food was twice as great following a rain than just prior to the rain. The content prior to the rain was entirely aquatic in source while that following the rain was entirely terrestrial (TABLE 5).

A comparison may be made between food intake of gar under field conditions with natural food available and under experimental conditions in which they were held in the presence of highly vulnerable food forms. Thus, the average percent of stomachs containing food in the wild was 38, while in the presence of crayfish and tadpoles 89 percent contained food (TABLE 5).

DISCUSSION

The ability of the shortnose gar to catch forage organisms is similar to

TABLE 4.—Seasonal Variation in the Food of Shortnose Gar in the Big Muddy River During 1966.

	Percent Occurrence					
	Fishes	Terr. insects	Aquatic insects	Benthos	Crayfish	Misc.
March	0	23	67	0	0	0
April	0	42	15	43	0	0
May	24	43	17	0	16	0
June	8	35	8	47	2	0
July	36	4	12	0	12	36[1]
August	47	29	0	18	0	6
September	52	14	9	19	5	1
October	100	0	0	0	0	0

[1] Plant material and debris.

that of the largemouth bass and channel catfish. In the pond environment, organisms that had been previously classed as vulnerable were most heavily utilized. The non-vulnerable forms, although readily utilized in the confinement of tanks, were poorly utilized in the ponds even when offered exclusively.

Some degree of adaptation and specialization in feeding of the shortnose gar is evident from the data. In tanks there was evidence of preference for the bullfrog tadpole. This organism is not palatable to the channel catfish (Anthony, 1964); it is, however, readily utilized by the largemouth bass. The extent to which the gar utilized naiads of the burrowing mayfly in the river environment suggests that the "beak" of the gar could be an adaptation for feeding on benthic forms, perhaps including infauna. Stomachs of largemouth bass of similar size collected along with the gar and apparently from the same habitat did not contain naiads of the mayfly.

The pronounced seasonal change in the stomach contents of the gar indicates considerable adaptability and,

TABLE 5.—Effects of Heavy Rainfall on the Percent of Full Stomachs and Occurrence of Terrestrial Food Items in Stomachs of the Shortnose Gar.

Sampling conditions	Percent containing food	Percent occurrence terrestrial
All samples	38[1]	22
Sample preceding rain	33	0
Sample after rain	77	77
Vulnerable forage in ponds	89[2]	....

[1] In comparison, Holloway (1954) reported 34 percent of 217 stomachs of shortnose gar sampled in July and August to contain food.

[2] Based on 42 shortnose gar held in ponds with tadpoles and crayfish.

of course, reflects seasonal availability.

Rainfall is shown to have a pronounced effect on food intake. The percent of stomachs containing food was twice as great following a rain. The composition also changed from no terrestrial food before the rain to 100 percent after.

The gar in the river were apparently not feeding at their maximum rate. The percent of stomachs containing food was only 38 as compared with 89 percent for gar in ponds with vulnerable forage organisms. Lewis (1967) has indicated that the food intake of predacious fishes in the wild may typically be minimal. Their maximum intake possibly is several times greater. Variations in rate of growth are associated with this variation in food intake.

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Manuscript received January 9, 1968.

CONTRIBUTIONS TO A FLORA OF ILLINOIS NO. 3. LEMNACEAE

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ABSTRACT. — The aquatic monocot family Lemnaceae is represented by four genera in Illinois: *Spirodela* Schleiden, *Lemna* L., *Wolffiella* Hegelmaier, and *Wolffia* Horkel. A recent survey of Illinois material reveals that these genera encompass fourteen species. Each species is described and its present distribution in the state is indicated.

The aquatic family Lemnaceæ has presented serious taxonomic difficulties for many years. The family has been monographed several times (Schleiden, 1839; Hegelmaier, 1868; Daubs, 1965), and a significant revision of the North American taxa excluding Mexico was completed by Thompson in 1898. Despite the existence of these major contributions, several closely related species remain recognized only questionably.

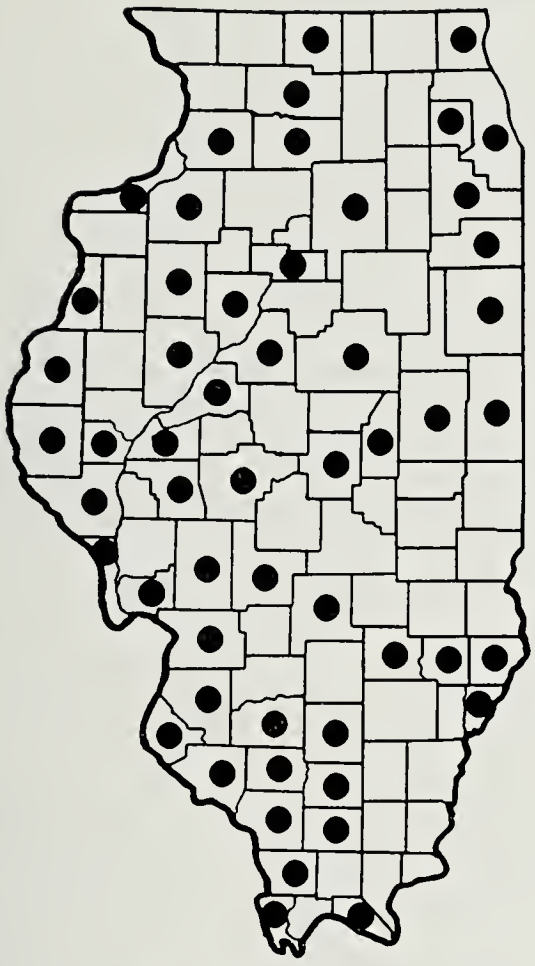
The family is cosmopolitan, usually considered to be comprised of four genera, all of which occur in Illinois. Some workers would combine *Lemna* with *Spirodela*, while Hutchinson (1959) does not recognize *Wolffiella*. It appears to us, however, that the maintenance of the four traditional genera (*Lemna, Spirodela, Wolffia*, and *Wolffiella*) most accurately depicts the situation in Illinois.

Flowering and fruiting specimens are extremely rare in collections and, when they do occur, are so reduced as to offer few characters of classificatory value. Thus, the delimitation of both the genera and the species rests heavily on vegetative features. It is partly because of this, since vegetative characters are variable, that taxonomic difficulties occur in the family.

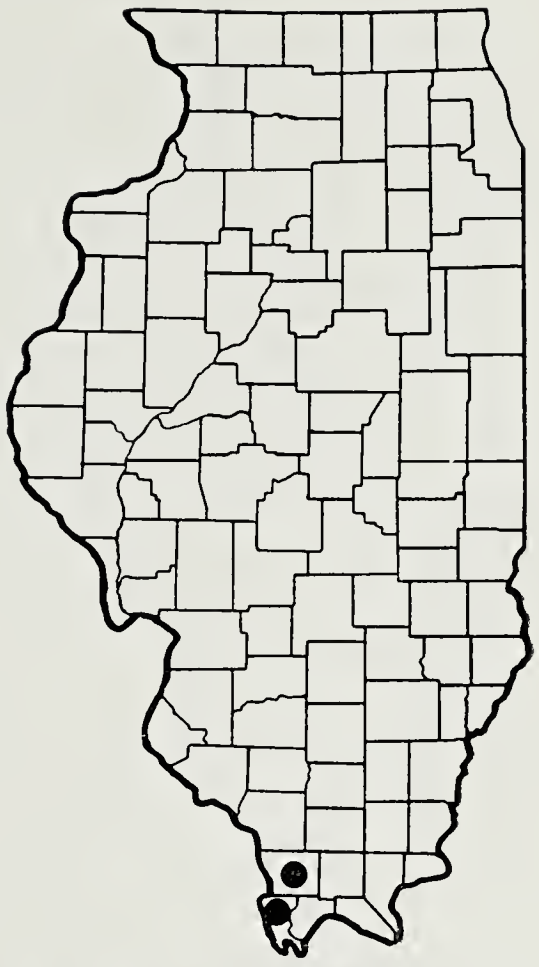
We are particularly disturbed in the genus *Lemna* where five of the species are separated only tenuously from each other. However, biochemical data of McClure and Alston (1966) indicate these difficult taxa (*L. minor, L. gibba, L. trinervis, L. minima*, & *L. obscura*) are distinct.

The student of duckweeds is further dismayed by the difficulty encountered in properly identifying dried herbarium specimens. The many obscure diagnostic features of fresh specimens become all but lost upon drying. A more desirable method of storing these plants is to place them in an aqueous preservative containing alcohol and formalin. Not only are aqueous preserved specimens superior to dried specimens in retaining useful diagnostic features, but a large number of them can be easily placed in a relatively small container.

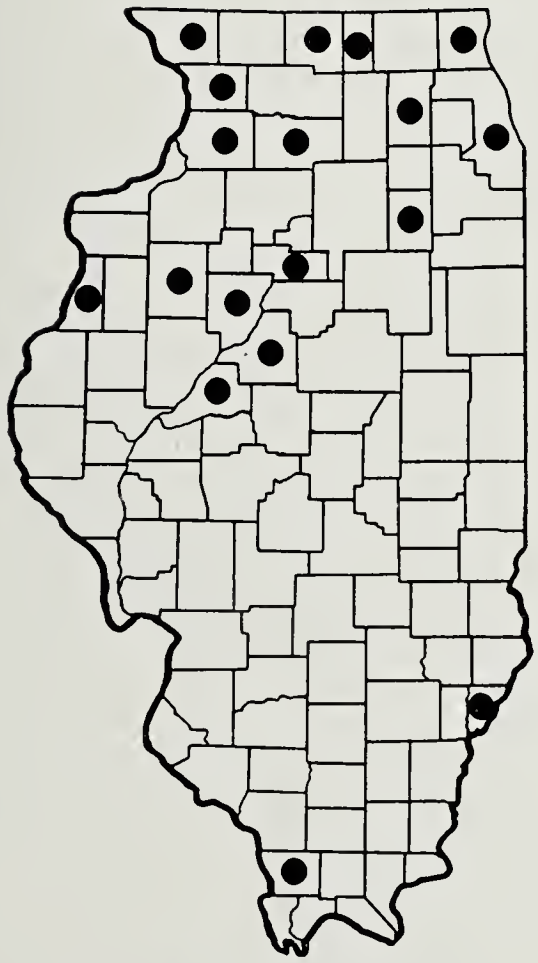
The question arises concerning the proper application of the much used



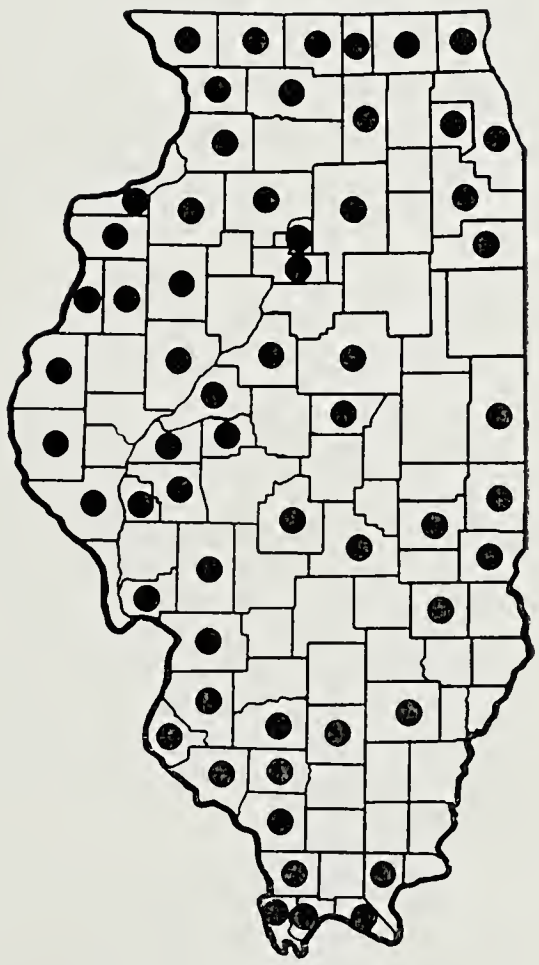
1. S. polyrhiza



2. S. oligorhiza



3. L. trisulca



4. L. minor

FIGURE 1.—Distribution records in Illinois for: SPIRODELA POLYRHIZA (L.) Schleiden, Map 1; SPIRODELA OLIGORHIZA Thompson, Map 2; LEMNA TRISULCA L., Map 3; LEMNA MINOR L., Map 4.

term "frond" in describing duckweeds. In the present study, frond is employed when speaking of the thalloid portion of the plant not including rootlets, flowers, and fruits. The term "plant" is more appropriately used when referring to the latter organs and the frond as a unit.

We have arbitrarily referred to the upper surface of the frond or plant as the dorsal surface, and to the lower surface (most commonly submerged) as the ventral surface.

This revision was only possible through the kind assistance given by curators of the following herbaria: Field Museum of Natural History (F); Illinois Natural History Survey (ILLS); Illinois State Museum (ISM); Missouri Botanical Garden (MO); University of Illinois (ILL); and Southern Illinois University (SIU). Appreciation is also expressed to those who collected specimens for the study and to Dr. J. W. McClure, Miami University, Oxford, Ohio, for supplying living plants of *Lemna gibba*.

Family Lemnaceae — Duckweed Family

Free-floating thalloid plants with 0-several ventral rootlets originating at the node; fronds nerved or nerveless; vegetative buds with flowers produced at the node in lateral, basal, or dorsal reproductive pouches; plants monoecious; flowers, in some species, surrounded by a spathe; staminate flowers 1-2 (-3), the anthers 1- to 2-celled; pistillate flowers 1, the ovary 1-celled, with 1 to several ovules; fruit a utricle, frequently ribbed; seed smooth or ribbed.

Key to the Genera of Lemnaceae in Illinois

1. Rootlets 1-several per plant; reproductive pouches lateral, 2 per frond.
   2. Rootlets (1-) 2 or more per plant ..... 1. *Spirodela*
   2. Rootlet 1 per plant ..... 2. *Lemna*
1. Rootlets none; reproductive pouch basal, 1 per plant.
   3. Frond thin, linear .... 3. *Wolffiella*
   3. Frond thick, globose or ellipsoid ..... 4. *Wolffia*

1. *Spirodela* Schleiden

Fronds flattened, orbicular to reniform, usually asymmetrical, 3- to 8-nerved; rootlets (1-) 2-10, arising in a fascicle beneath the node; reproduction from two lateral pouches on either side of the node; flowers and fruit infrequently produced; ovules 2.

Key to the Species of *Spirodela* in Illinois

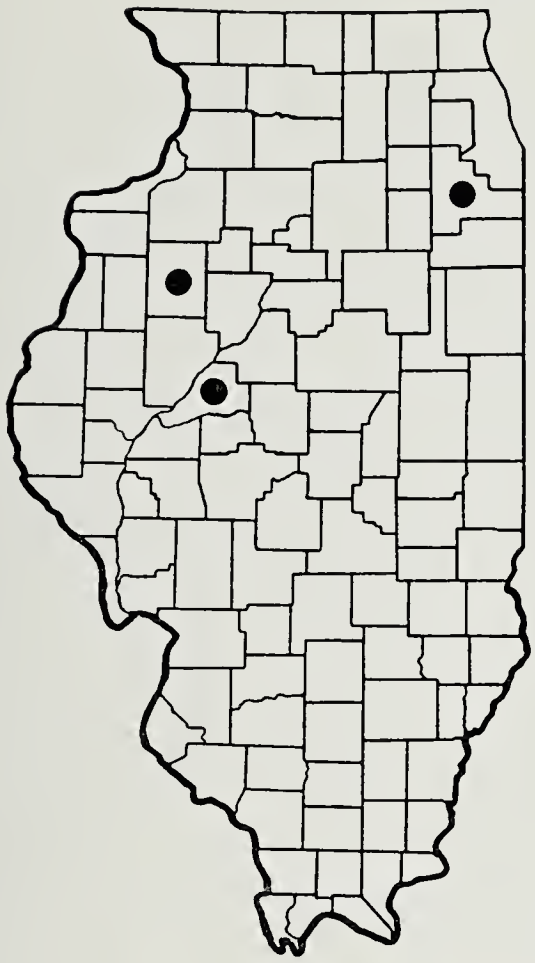
1. Fronds orbicular, 5- to 8-nerved; rootlets 2-10 ..... 1. *S. polyrhiza*
1. Fronds obovate to reniform, obscurely 3-nerved; rootlets (1-) 2-3 ..... 2. *S. oligorhiza*

1. SPIRODELA POLYRHIZA (L.) Schleiden, Linnaea 13:392. 1839. Map 1. Fig. 5 (a,b,&c).

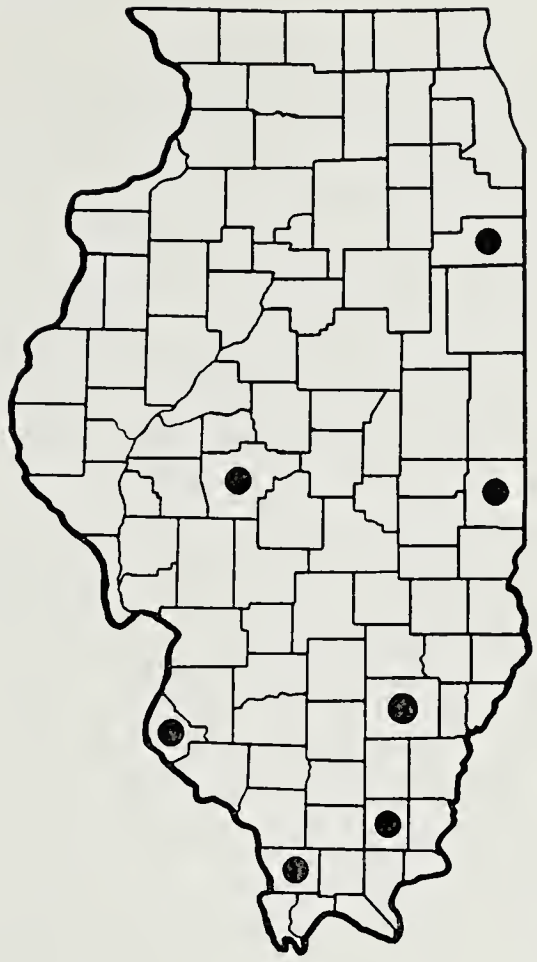
Lemna polyrhiza L. Sp. Pl. 970. 1753.
Lemna major Griff. Notul. 3:216. 1851.

Fronds orbicular to obovate (immature fronds orbicular), weakly to strongly asymmetrical, apiculate, 2-7 mm long, 2-6 mm wide, weakly inflated, cavernous throughout, prominently 5- to 8-nerved, the nerves originating palmately at the node, lateral nerves strongly incurved toward the central nerve after departure from the node; upper surface flattened, often olive-green, guard cells abundant, with a row of papules frequently present along the central nerve and occassionally along lateral nerves; lower surface flattened or weakly convex, pale green or occasionally reddish-purple; rootlets 2-10, arising in a fascicle directly beneath the node on the lower surface, up to 15 mm long, rootcaps acute; plants solitary or commonly remaining attached in groups of 2-5; vegetatively produced plants and infrequently observed flowers arising from two lateral, reproductive pouches; fruit a somewhat compressed and rounded utricle with winged margins; seed smooth or only slightly ribbed.

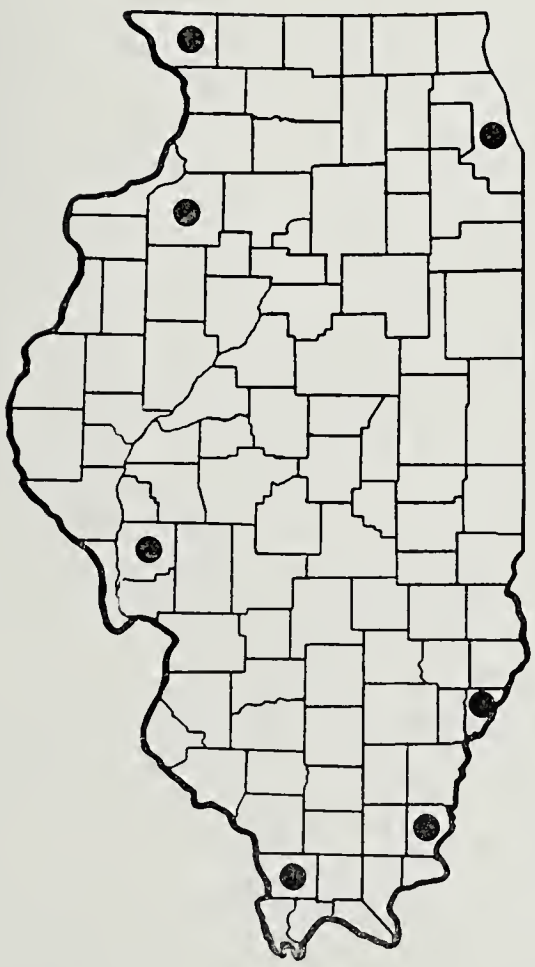
This species is variable in both shape and thickness, but despite this variability is easily separated from the other species upon critical examination of nerve and rootlet numbers. The frond of *S. polyrhiza* is also generally larger than that of *S. oligorhiza*.



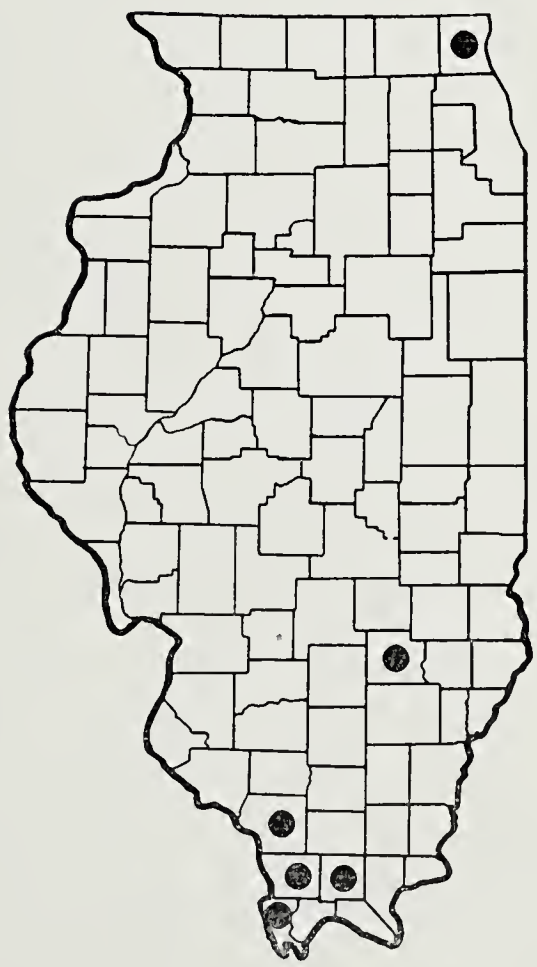
5. L. gibba



6. L. perpusilla



7. L. trinervis



8. L. valdiviana

FIGURE 2.—Distribution records in Illinois for: LEMNA GIBBA L., Map 5; LEMNA PERPUSILLA Torrey, Map 6; LEMNA TRINERVIS (Austin) Small, Map 7; and LEMNA VALDIVIANA Philippi, Map 8.

The nodal region on the upper surface of the frond is often red-pigmented and has been referred to by various authors as the "eyespot." According to Hicks (1937), this condition is most prevalent in fronds growing in the sun.

Spirodela polyrhiza is undoubtedly one of the most widely distributed members of the family. It occurs throughout North America, as well as in Europe, Asia, and tropical America. It probably occurs in every Illinois county.

Specimens Examined: ADAMS: Quincy Bay, *Evers 144* (ILL). ALEXANDER: E. of Miller City, *Evers 30425* (ILLS). BROWN: LaGrange, *Rexroat 5829* (ISM). CALHOUN: Six miles N.E. of Belleview, *Evers 32408* (ILLS). CASS: N. of Virginia, *Winterringer 5143* (ISM). CHAMPAIGN: Salt Fork, *Jones 17380* (ISM). CLAY: N. of Louisville, *Evers s.n.* (ILLS). COOK: Stoney Brook, Blue Island, *Chase s.n.* (ILL). DUPAGE: DuPage River at Naperville, *Umbach s.n.* (F). FAYETTE: E. of Vandalia, *O'Dell 508* (ILL). FRANKLIN: One mile W. of Zeigler, *Fore & Stookey 660* (SIU). FULTON: Anderson Lake, *Evers & Mills 33050* (ILLS). HANCOCK: Mississippi River, Pontoosuc, *Kibbe 9597* (ILLS). HENDERSON: Mississippi River, two miles S. of Lomax, *Dolbeare 219* (ISM). HENRY: Illinois & Mississippi Canal, *Dobbs 19543* (ILLS). IROQUOIS: Watseka, *Jones s.n.* (ILL). JACKSON: Campbell Lake, *Bailey & Swayne 2672* (SIU). JEFFERSON: L & S Lake, *Fore & Stookey 748* (SIU). JERSEY: W. of Grafton, *Evers 38867* (ILLS). KANKAKEE: Farr's Woods, *De Selm 676* (F). KNOX: Lake Storey, *Evers 31821* (ILLS). LAKE: Lake Villa, *Fuller 6870* (ISM). LA SALLE: Starved Rock, *Werner 7614* (ISM). LAWRENCE: Allison Creek, *Fisher s.n.* (ISM). LEE: Amboy, *Long 975* (ILL). McLEAN: Lake Bloomington, *Weik 174* (SIU). MACON: N.E. of Decatur, *Evers 38680* (ILLS). MACOUPIN: Rinaker Lake, *Adams 9600* (ILLS). MADISON: W. of Marine, *Winterringer 3898* (ISM). MARSHALL: Near Henry, *Meek s.n.* (F). MASON: Two miles W. of Forest City, *Evers 30925* (ILLS). MASSAC: Mermet Lake, *Weik B139* (SIU). MONROE: N. of Burksville, *Evers 32777* (ILLS). MONTGOMERY: Big Four Reservoir, *Lockhart s.n.* (ISM). MORGAN: Waverly Lake, *Carter 14357* (ISM). OGLE: Byron, *Blount 235* (ISM). PEORIA: Illinois River, Peoria, *Heading s.n.* (ILL). PERRY: Reese Creek, E. of Old DuQuoin, *Evers 32714* (ILLS). PIATT: *Waite 18351* (ILL). PIKE: N. of Pike, *Evers & Mills 33037* (ILLS). RANDOLPH: N. of Prairie Du Rocher, *Evers 32782* (ILLS). RICHLAND: One mile N. of Olney, *Weik 156* (SIU). ROCK ISLAND: Andalusia Slough, *Evers 39686* (ILLS). ST. CLAIR: Seven miles E. of Dupo, *Fuller 13588* (ISM). SANGAMON: Sangamon River, *Hall s.n.* (ILL). TAZEWELL: Illinois River, *Chase 6169* (ILL). UNION: Wolf Lake, *Ahles 2866* (ILL). VERMILION: One-fourth mile E. of Muncie, *Storm s.n.* (ILL). WABASH: Mauck's Pond, Mt. Carmel, *Schneek s.n.* (ILL). WASHINGTON: Muddy Lake, *Evers 39103* (ILLS). WHITESIDE: N.W. of Malvern, *Winterringer 2896* (ISM). WILL: Des Plaines River, *Hill 41.1907* (ILL). WILLIAMSON: Crab Orchard Lake, *Evers 30491* (ILLS). WINNEBAGO: Snow Slough, Rock River, *Thompson & Tehon 9595* (ILLS).

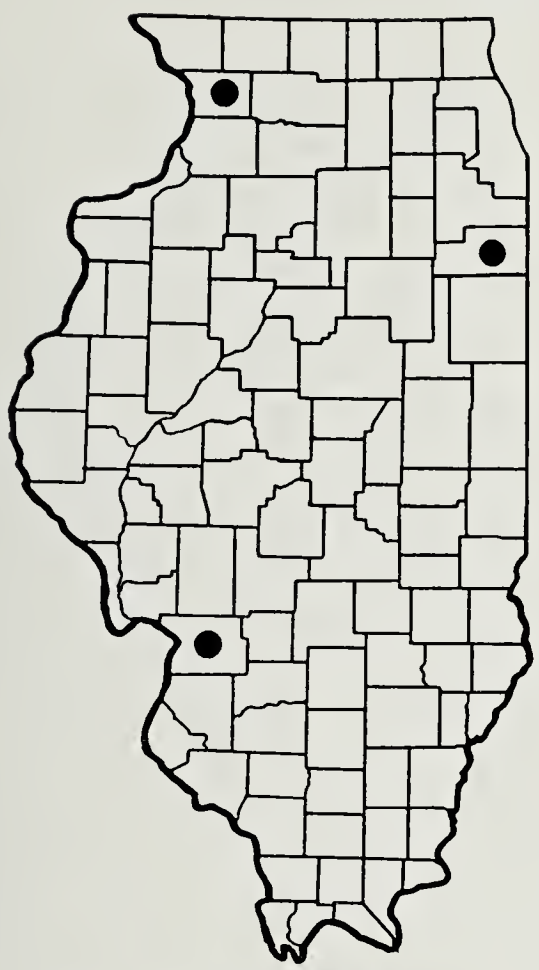
2. SPIRODELA OLIGORHIZA (Kurz) Hegelm. Die Lemnaceen 147. 1868. Map 2. Fig. 5. (d, e, & f).

Lemna oligorhiza Kurz, Journ. Limn. Soc. London 9:267. 1867.

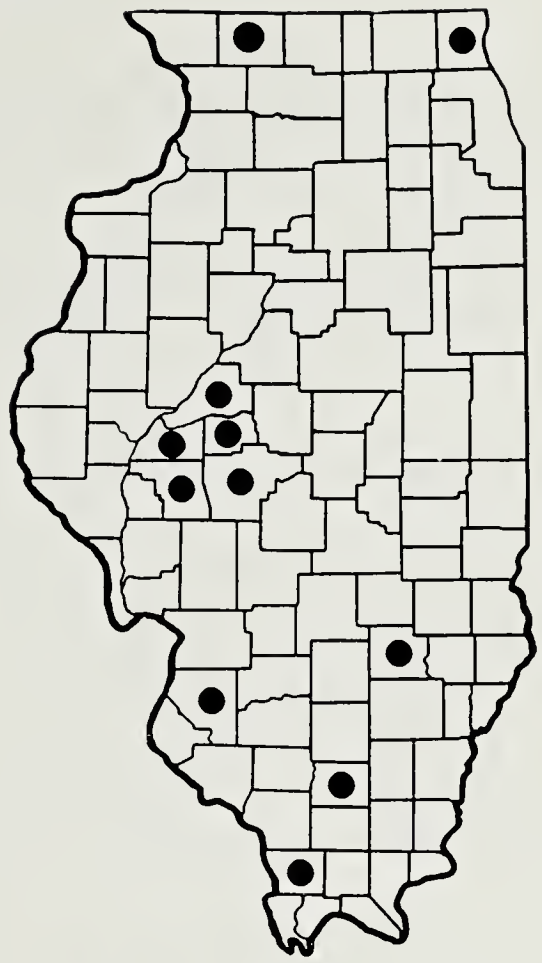
Fronds ovate to reniform, asymmetrical, subacute to obtuse at the apex, 2.2-6.0 mm long, 2.0-2.6 mm wide, inflated, cavernous throughout, obscurely 3- (5-) nerved; upper surface convex, sparsely punctate with pigment cells, guard cells abundant; lower surface weakly convex, somewhat darker than the upper; rootlets (1-) 2-5, arising in a darkened fascicle, directly beneath the node; rootcaps obtuse; plants solitary or commonly remaining attached in groups of 2-3; vegetatively produced plants and infrequently observed flowers arising from two lateral, reproductive pouches; fruit slightly winged, ovoid; seeds longitudinally ribbed.

Spirodela oligorhiza is easily distinguished from *S. polyrhiza*, but the inexperienced student might mistake it for a member of the genus *Lemna* since at times it may possess only a single rootlet.

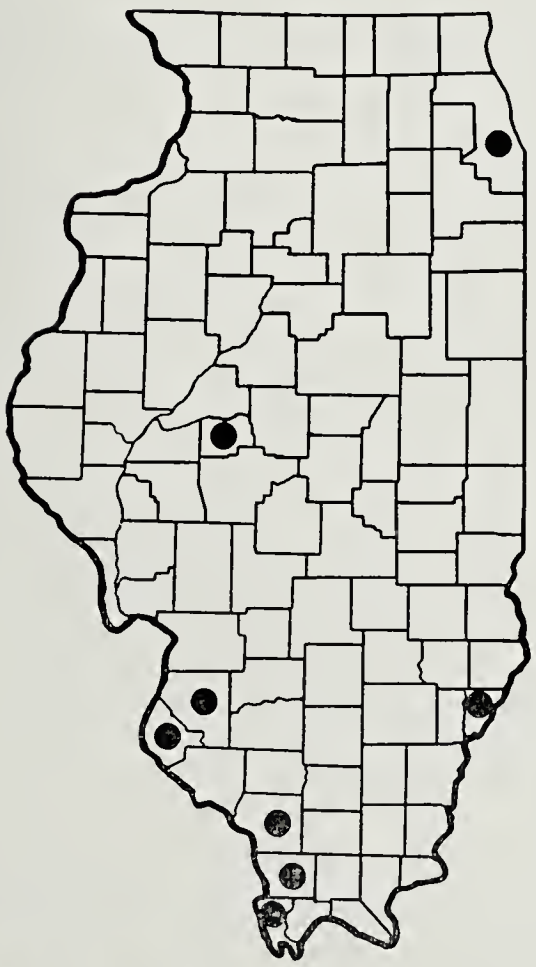
The biochemical data of McClure and Alston (1966) strongly suggest a close relationship between *S. oligorhiza* and *L. minor*. This relationship was recognized by Hegelmaier, also (1896). Future evidence may necessitate a more realistic positioning of this species in the genus *Lemna*.



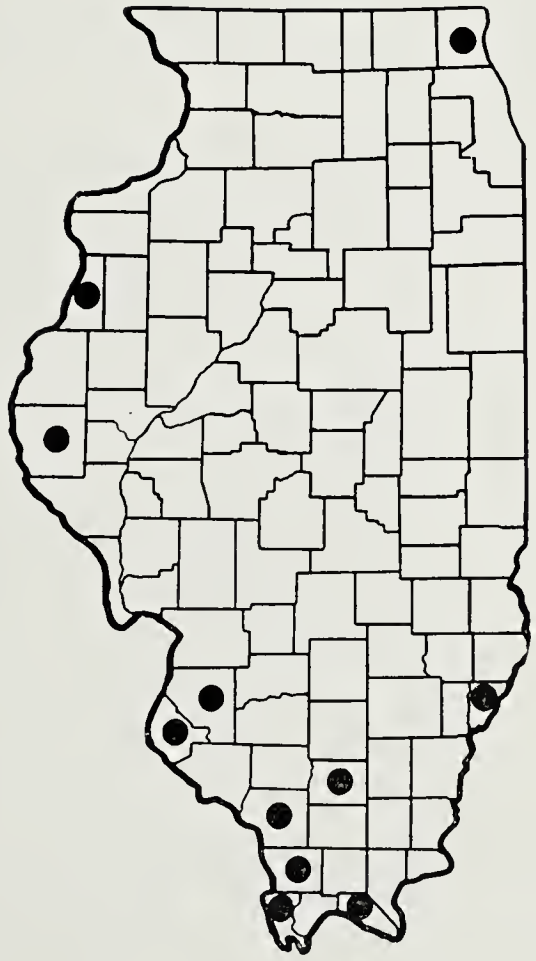
9. L. minima



10. L. obscura



11. Wf. floridana



12. W. papulifera

FIGURE 3.—Distribution records in Illinois for: LEMNA MINIMA Philippi, Map 9; LEMNA OBSCURA (Austin) Daubs, Map 10; WOLFFIELLA FLORIDANA (J. D. Smith) Thompson, Map 11; and WOLFFIA PAPULIFERA Thompson, Map 12.

This species is thought to be native to the Orient, having been introduced sparingly in the United States. In Illinois, it is known only from Alexander and Union counties.

Specimens Examined: ALEXANDER: Horseshoe Lake, *Weik 72* (SIU). UNION: La Rue Swamp, *Hopkins A51* (SIU).

2. *Lemna* L. — Duckweed

Fronds flattened to strongly convex on the ventral surface, orbicular to spatulate, symmetrical to asymmetrical, occasionally long-stipitate, 1- to 3- (5-) nerved, the nerves frequently obscure; rootlet 1, the rootcap acute to obtuse; reproduction from two lateral pouches on either side of the node; flowers and fruit sparingly produced; ovules 1-6.

Key to the Species of *Lemna* in Illinois

1. Fronds spatulate with long, persistent stipes, often submerged in compact masses; rootlet frequently absent .....1. *L. trisulca*
1. Fronds orbicular to elliptic, floating; rootlet present.
   2. Fronds 3- to 5-nerved.
      3. Root sheaths cylindrical.
         4. Lower surface of frond flattened or only weakly convex, apex rounded to acute, frond usually 3-nerved .....2. *L. minor*
         4. Lower surface of frond moderately to strongly convex, apex broadly rounded or often obtuse, frond 3- to 5- nerved..... .....3. *L. gibba*
      3. Root sheaths winged.
         5. Fronds weakly to strongly asymmetrical, thick, the nerves often inconspicuous .....4. *L. perpusilla*
         5. Fronds symmetrical or nearly so, membranous, the 3 nerves prominent .....5. *L. trinervis*
   2. Fronds 1-nerved or obscurely nerved.
      6. Fronds 1-nerved, the lower surface green.
         7. Fronds ovate-elliptic to elliptic, weakly to moderately asymmetrical, often floating in compact masses ....6. *L. valdiviana*
         7. Fronds obovate to orbicular, symmetrical, usually solitary .....7. *L. minima*
      6. Fronds obscurely nerved, the lower surface frequently reddish-purple .....8. *L. obscura*

1. LEMNA TRISULCA L. Sp. Pl. 970. 1753. Map 3. Fig. 5 (g & h).

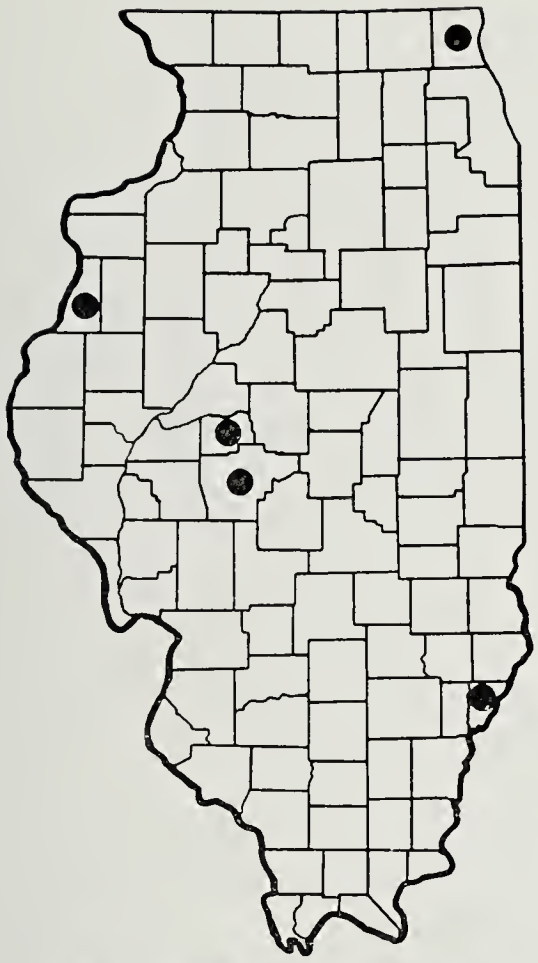
Fronds elliptic to oblanceolate, commonly falcate with long-tapering stipes, 4-10 mm long, 1.5-3.0 mm wide, membranous, obscurely 3-nerved, the apical margin serrulate; upper surface flattened, dull green, guard cells absent; lower surface flattened; rootlets normally lacking, if present, the length highly variable, at times weakly coiled, the sheath winged, the rootcap long, obtuse; plants attached to one another at the nodes by elongated stipes, rarely solitary, generally submerged; fruit symmetrical; seeds longitudinally ribbed with numerous cross-striae.

This species is often referred to as star duckweed or submerged duckweed because attached plants frequently produce stellate colonies which are normally submerged. It is the most easily recognized *Lemna* in Illinois where it is known throughout the state but is not widespread.

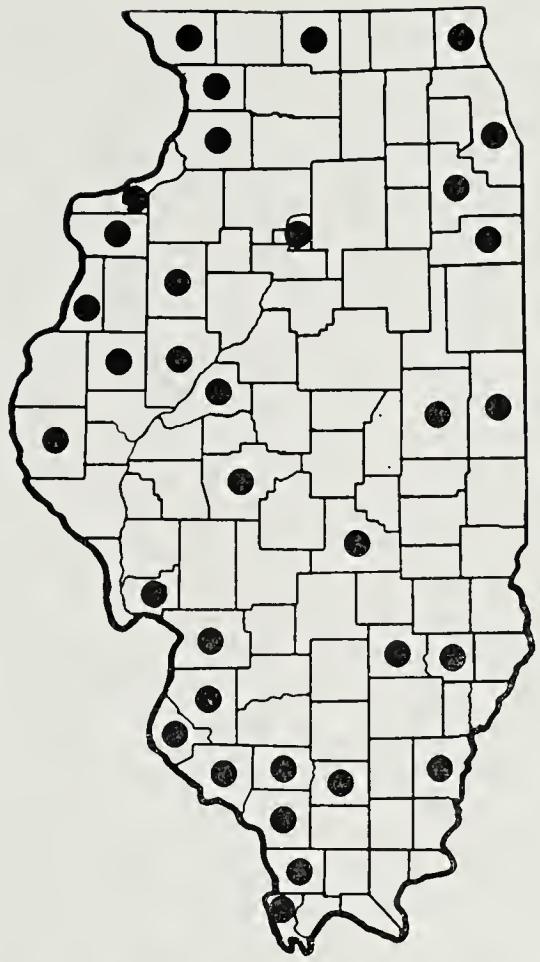
The overall range of *L. trisulca* includes every continent except South America and Australia.

Specimens Examined: BOONE: Kishwaukee River Slough, W. of Belvidere, *Fell f45739* (ISM). CARROLL: Two miles N. of Savannah on Rt. 84, Palisades State Park, *Rock s.n.* (ISM). COOK: N. of Palatine, *Evers 34839* (ILLS). GRUNDY: Near Morris, *Vasey 1* (ISM). HENDERSON: Oquawka, *Patterson s.n.* (MO). JO DAVIESS: 8 miles S. of Galena, *Roek s.n.* (ISM). LAKE: Cedar Lake, Lake Villa, *Fuller 13262* (ISM). LEE: Near Amboy, *Chase 12412* (ILL). MARSHALL: *Chase 10684* (ISM). MASON: Pond, on Salt Creek, *Hall* (MO). PEORIA: Peoria, *Brendel s.n.* (ILL). TAZEWELL: Spring Mill bog, *Chase 9069* (ISM). UNION: Wolf Lake, *Swayne* (ILL). WABASH: Mt. Carmel, *Schneck 261* (ILL). WHITESIDE: 3 miles W. of Prophetstown, *Tehon 9585* (ILLS). WINNEBAGO: Shallow water, Killbuck Creek, S. of Rockford, *Fell 52465* (ISM).

2. LEMNA MINOR L. Sp. Pl. 970. 1753. Map 4. Fig. 6 (a,b,c,d,&e).



13. W. punctata



14. W. columbiana

FIGURE 4.—Distribution records in Illinois for: WOLFFIA PUNCTATA Grisebach, Map 13; and WOLFFIA COLUMBIANA Karsten, Map 14.

Fronds obovate to elliptical (immature fronds orbicular), symmetrical or weakly asymmetrical, 2-5 mm long, 1.5-3.5 mm wide, flattened to weakly inflated, cavernous throughout, 3-nerved, the nerves occasionally obscure; upper surface usually slightly convex, with a thick, glistening cuticle, guard cells abundant, a median row of papules commonly present; lower surface flattened or weakly to moderately convex, pale green or occasionally reddish-purple; rootlet one, arising obliquely beneath the node and lying in a narrow furrow, its length highly variable, the sheath frail, cylindrical, the rootcap obtuse; plants solitary or commonly remaining attached in groups of 2-4; fruit symmetrical, broadly ovoid; seeds longitudinally ribbed.

This species is apparently quite variable in both size and coloration. Large specimens have been observed up to 5 mm in length. These types are usually somewhat flattened and membranous. Other specimens have been observed with an obvious reddish-purple pigmentation on the lower surface. This condition appears to be associated with the smaller plants which also usually have conspicuously inflated fronds. The latter specimens are often quite difficult to separate from *L. obscura*; however, careful examination normally reveals three obscure nerves.

Lemna minor occurs throughout North America, Europe, Asia, Africa, and Australia. It is the most widely distributed species of *Lemna* in Illinois.

Specimens Examined: ADAMS: Quincy Bay, *Evers 144* (ILLS). ALEXANDER: Horseshoe Lake, *Evers 30449* (ILLS). BOONE: Six miles W. of Belvidere, *Tichacek & Muench s.n.* (ISM). BUREAU: Illinois & Mississippi Canal near Buda, *Daubs 860* (ILL). CARROLL: *DeFilipps & Ozment 115* (SIU). CASS: S. of Beardstown, *Evers 30881* (ILLS). CHRISTIAN: N.E. of Morrisonville, *Evers 31056* (ILLS). CLARK: Five miles E. of Marshall, *Fritz s.n.* (ISM). COLES: *Stotler s.n.* (SIU). COOK: Skokie Marsh W. of Glencoe, *Evers 32221* (ILLS). DE KALB: Three miles N.W. of Sycamore, *Tichacek & Muench s.n.* (ISM). DE WITT: Weldon Springs State Park, *Weik 307* (SIU). DU PAGE: Five miles S. of Downers Grove, *Evers 32147* (ILLS). EDGAR:

S. of Vermilion, *Evers 31540* (ILLS). FULTON: Anderson Lake, *Evers 33051* (ILLS). HANCOCK: Mississippi River, two miles S. of Nauvoo, *Dolbeare s.n.* (ISM). HENDERSON: N. of Bald Bluff, *Evers 32000* (ILLS). HENRY: Crescent Lake, *Russell s.n.* (ISM). JACKSON: Lake Murphysboro, *Mohlenbroek A6* (SIU). JASPER: *Weik 158* (SIU). JEFFERSON: *Fore & Stookey 746* (SIU). JERSEY: Lake W. of Grafton, *Evers 38869* (ILLS). JO DAVIESS: Mississippi River S. of Blanding, *Evers 35763* (ILLS). KANKAKEE: N.W. of Wichert, *Evers 18510* (ILLS). KNOX: Horseshoe Lake, *Weik B109* (SIU). LAKE: Volo Bog, *Evers 34934* (ILLS). LA SALLE: Starved Rock State Park, *Daubs 876* (ILL). McHENRY: Marsh N. of Wonder Lake, *Evers 69124* (ILLS). McLEAN: Bloomington, *Jones s.n.* (ILL). MACOUPIN: Standard City Lake, *Lopinot s.n.* (ISM). MADISON: Maryville Fishing Club Lake, *Lockhart s.n.* (ISM). MARSHALL: S. of Lacon, *Evers 19601* (ILLS). MASON: One mile N. of Forest City, *Evers s.n.* (ILLS). MASSAC: Mermet Lake, *Weik 298* (SIU). MENARD: Athens, *Hall 18346* (ILL). MERCER: N. of Keithsburg, *Evers 31943* (ILLS). MONROE: N. of Burksville, *Evers 32776* (ILLS). MORGAN: Morgan Lake, *Rodgers s.n.* (ISM). OGLE: Illinois Central Sportsman Club Lake, *Rock s.n.* (ISM). PERRY: N. of Conant, *Evers 38947* (ILLS). PIKE: W. of Meredosia, *Rexroat 6517* (ISM). POPE: N. of Temple Hill, *Ozment s.n.* (SIU). PULASKI: *Fore & Stookey s.n.* (SIU). PUTNAM: *DeFilipps & Ozment 11* (SIU). RANDOLPH: N. of Chester, *Evers 32785* (ILLS). ROCK ISLAND: N.W. of Milan, *Evers 31907* (ILLS). ST. CLAIR: Campus of St. Henry College, Belleville, collector not cited (ILLS). SCOTT: *DeFilipps & Ozment 2* (SIU). SHELBY: Two & one-half miles N.W. of Windsor, *Fritz s.n.* (ISM). STEPHENSON: *DeFilipps & Ozment 36* (SIU). TAZEWELL: Illinois River, *Chase 6170* (ILL). UNION: 4 miles S. of Ware, *Evers 32797* (ILLS). VERMILION: One-half mile S. of Muncie, *Morris s.n.* (ISM). WARREN: *DeFilipps & Ozment 146* (SIU). WASHINGTON: S.W. of Venedy Station, *Evers 39104* (ILLS). WAYNE: Elm River slough N.E. of Fairfield, *Fisher s.n.* (ISM). WHITESIDE: Three miles W. of Prophetstown, *Thompson & Tchon 112* (ILLS). WILL: DuPage River three miles N. of Plainfield, *Bergseng s.n.* (ILL). WINNEBAGO: N. of Winnebago, *Evers 35375* (ILLS).

3. LEMNA GIBBA L. Sp. Pl. 970. 1753. Map 5. Fig. 6 (f,g,h,i,j,k&l).

Fronds orbicular to cuneate, symmetrical to weakly asymmetrical, 2-6 mm long, 1-4 mm wide, commonly inflated and cavernous throughout especially on the lower surface, obscurely 1- to 3-(5-) nerved; upper surface flat to weakly convex, the guard cells abundant, commonly with a median row of papules and occasionally keeled, dark green or sometimes purple; lower surface flattened to strongly gibbous; rootlet one, the sheath cylindrical, prominent, the rootcap obtuse; plants solitary or commonly remaining attached in groups of 2-4 by short to somewhat elongated stipes; fruit winged, depressed-globoid; seeds 2-4, compressed, asymmetrical, longitudinally ribbed.

The first collection of this duckweed in Illinois was apparently made in Mason County by T. W. Burrill in 1894. Although Burrill's specimens are dried, their strong resemblance to living plants of *L. gibba* is unquestionable. The specimens of McClure and Daubs leave no doubt concerning the authenticity of its occurrence in Illinois.

Upon drying, specimens of *L. gibba* may collapse enough to superficially resemble *L. minor* or *S. polyrhiza*.

Lemna gibba occurs on every major continent with the exception of Australia. In Illinois specimens are known only from Knox, Mason, and Will counties.

Specimens Examined: KNOX: Thirteen miles N. of Elmwood, *McClure 46* (SIU). MASON: Quiver Lake, Havana, *Burrill s.n.* (ILL). WILL: Des Plaines River near Dresden Locks, *Daubs 877* (ILL).

4. LEMNA PERPUSILLA Torr. Fl. N.Y. 2:245. 1843. Map 6. Fig. 6 (m,n,o,p,q&r).

Fronds ovate to obovate, variable, asymmetrical, 1.5-3.0 mm long, 1.0-1.5 mm wide, inflated, obscurely 3-nerved or apparently nerveless, cavernous in the central region only; upper surface weakly convex, the guard cells abundant, with prominent nodal and apical papules and occasional smaller papules on median line between; lower surface flattened or weakly convex; rootlet one, with a winged sheath, the rootcap acute; plants solitary or commonly remaining attached in groups of 2-3; fruit asymmetrical, ovoid to oblongoid with a prominent

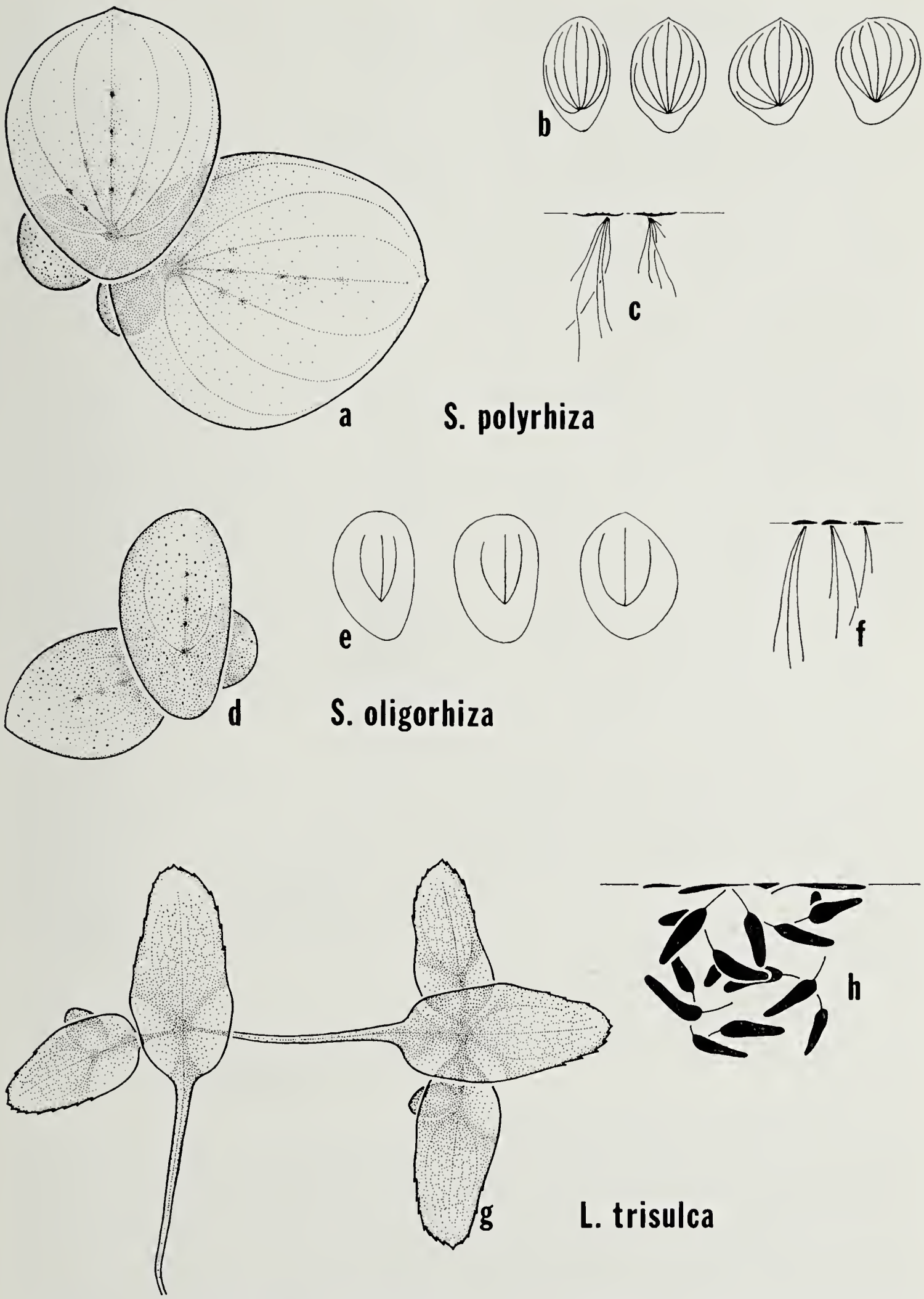


FIGURE 5.—SPIRODELA POLYRHIZA (L.) Schleiden, a. habit, dorsal aspect X5; b. morphological variation among mature fronds X2; c. floating habit, lateral aspect X1. SPIRODELA OLIGORHIZA Thompson, d. habit, dorsal aspect X5; e. morphological variation among mature fronds X2; f. floating habit, lateral aspect X1. LEMNA TRISULCA L., g. habit, dorsal aspect X5; h. floating habit X1.

oblique style directed toward the apex of the frond; seeds longitudinally ribbed and with numerous cross-striae.

The characteristic winged root sheath and asymmetrical frond beset with papules help to distinguish this duckweed from all others in Illinois. The winged root sheath can best be seen with the aid of a compound microscope.

Lemna perpusilla is known from Massachusetts to North Dakota, south to Kansas and Florida, as well as from California and South America. In Illinois, it has been collected in a few counties scattered throughout the state.

Specimens Examined: EDGAR: Paris West Lake, Paris, *Fritz s.n.* (ISM). KANKAKEE: N.E. of St. Anne, Kankakee, *Fuller 8960* (ILL). MONROE: Murdock Lake, *Fore & Stookey 640* (SIU). SALINE: Harrisburg, North Reservoir, *Fore & Stookey 583* (SIU). SANGAMON: Curran Twp., Sec. 34, *Fuller 6236* (ISM). UNION: E. of Wolf Lake, *Ahles 2566* (ILL). WAYNE: One mile E. of Fairfield, *Fore & Stookey 728* (SIU).

5. LEMNA TRINERVIS (Austin) Small, Fl. S.E. U.S. 230. 1903. Map 7. Fig. 7 (a,b,c,d,&e).

Lemna perpusilla var. *trinervis* Austin in Gray, Man. Bot. 479. 1867.

Fronds obovate to ovate-elliptic, symmetrical, 2.5-4.0 mm long, 1.5-2.0 mm wide, relatively thin and membranous, prominently 3-nerved, weakly cavernous in the central region only; upper surface flattened, the guard cells abundant, an apical papule sometimes present; lower surface flattened; rootlet one, with a winged sheath, the rootcap acute; plants solitary or commonly remaining attached in groups of 2-3; fruit asymmetrical, broadly ovoid to oblongoid with a prominent oblique style directed toward the apex of the frond; seeds longitudinally ribbed with numerous cross-striae.

The morphological similarity between *L. perpusilla* and *L. trinervis* is obvious. Several important characteristics are shared by both taxa. The frond of *L. trinervis*, however, tends to be thin and membranous and its three nerves are very prominent. Its symmetry is normally regular, although at times it may exhibit a slight asymmetry more characteristic of *L. perpusilla*.

Lemna trinervis is restricted to North, Central and Southern America. In Illinois it occurs in several widely separated counties.

Specimens Examined: COOK: Chicago, *Nelson s.n.* (ILL). GALLATIN: Big Lake, *Fore & Stookey 575* (SIU). GREENE: Anna Keatch's Ranch, *Moffatt s.n.* (ILL). HENRY: Illinois & Mississippi Canal, *Dobbs 1954* (ILLS). JO DAVIESS: Ponds below East Dubuque, *Pepoon 9582* (ILLS). UNION: Pond E. of Wolf Lake, *Ahles 2980* (ILL). WABASH: Long Pond, Mt. Carmel, *Schneck s.n.* (ILL).

6. LEMNA VALDIVIANA Phil. Linnaea 33:239. 1864. Map 8. Fig. 7 (f,g,&h).

Lemna cyclostasa Ell. ex Thomp. Rep. Mo. Bot. Gard. 9:35 1898.

Fronds oblong to oblong-elliptic, asymmetrical and often distinctly falcate, 1-5 mm long, 0.5-2.0 mm wide, obscurely 1-nerved or nerveless, cavernous throughout; upper surface convex, usually pale green and glossy, with guard cells sparingly produced, a median row of minute papules occasionally present; lower surface flattened or weakly convex, pale green; rootlet one, the sheath long and cylindrical, the rootcap usually long and weakly reflexed, acute; plants usually remaining attached in groups of 2-4 or more, frequently forming dense masses; fruit asymmetrical, oblongoid; seeds longitudinally ribbed and with numerous cross-striae.

Lemna valdiviana may be distinguished from other members of the genus by its narrow, often falcate fronds and generally smaller size, although very small plants may occasionally resemble *L. minima*. McClure and Alston (1966), however, found these two taxa to be quite distinct in their flavonoid chemistry.

This species is known sparingly throughout the United States and from South America. Evers first collected it in Illinois in 1947 from Volo Bog in Lake County, but distribution is restricted chiefly to the southern third of the state.

Specimens Examined: ALEXANDER: Horseshoe Lake, *Weik 213* (SIU). CLAY: One-half mile N. of Louisville, *Fisher s.n.* (ISM). JACKSON: Campbell Lake, *Dixon 5* (SIU). JOHNSON: Cache River, S. of Cypress, *Evers 36038* (ILLS). LAKE: Volo Bog, *Evers 4670* (ILLS). UNION: Wolf Lake, *Bailey & Swayne 1166* (SIU).

7. LEMNA MINIMA Phil. Linnaea 33:239. 1864. Map 9. Fig. 7 (i,j,&k).

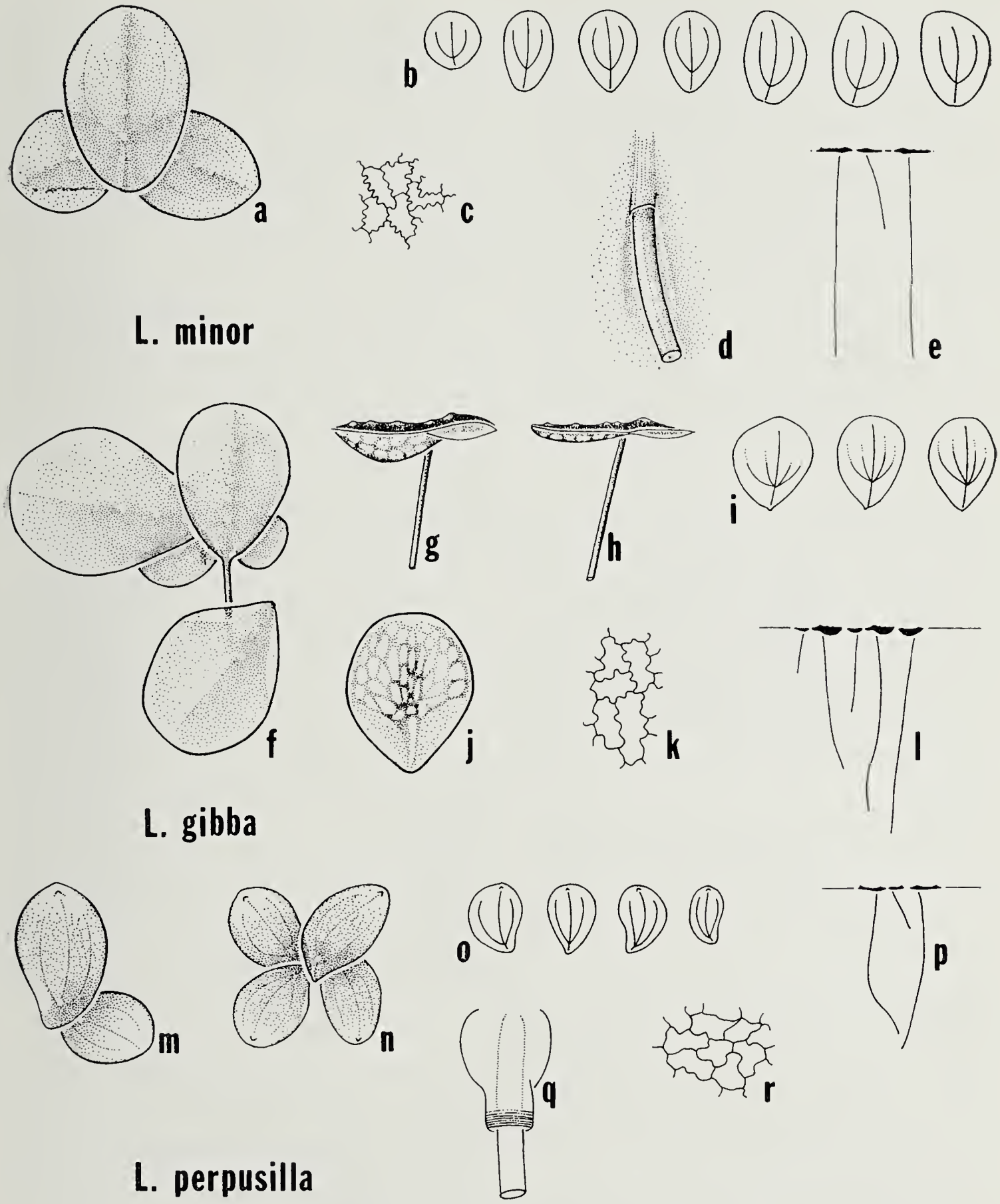


FIGURE 6.—LEMNA MINOR L., a. habit, dorsal aspect X5; b. morphological variation among mature fronds X2½; c. epidermal cell wall pattern, lower surface X200; d. origin of single rootlet showing characteristic furrow formed on lower surface X12½; e. floating habit, lateral aspect X1. LEMNA GIBBA L., f. habit, dorsal aspect X5; g. lateral view of a frond showing strong development of gibbous condition on lower surface X5; h. lateral view of a frond showing weak development of gibbous condition on the lower surface X5; i. morphological variation among mature fronds X2½; j. lower surface of a frond showing development of air spaces in strongly gibbous condition X5; k. epidermal cell wall pattern, lower surface X200; l. floating habit, lateral aspect X1. LEMNA PERPUSILLA Torrey, m. & n. habit, dorsal aspects X5; o. morphological variation among mature fronds X2½; p. floating habit X1; q. winged sheath surrounding rootlet at point of origin on lower surface X25; r. epidermal cell wall pattern, lower surface X200.

Lemna valdiviana var. *minima* (Phil.) Hegelm. Lemnac. 138. 1868.

Fronds obovate to elliptical, symmetrical to weakly asymmetrical, 1.5-1.7 mm long, 1.0-1.2 mm wide, weakly inflated, obscurely 1-nerved or apparently nerveless, cavernous only in the central region, thin-margined; upper surface convex, pale green, glossy, guard cells present, often with a median row of papules commonly present; lower surface flattened or weakly convex, pale green or yellowish; rootlet one, the sheath cylindrical, thin, the rootcap acute or obtuse; plants frequently solitary or remaining attached in groups of 2 (-4); fruit elongated, symmetrical; seeds longitudinally ribbed and with cross-striae.

This species probably presents the greatest difficulty for students of Illinois duckweeds. Its small size and poor condition upon drying contribute much to the problem of proper identification. It is often erroneously identified as *L. minor* or *L. perpusilla*. Jones (1963) circumvents this problem by combining this species with *L. minor* in the *Flora of Illinois*. The flavonoid chemistry of these two species is so distinct, however, that evidence is strongly in favor of retaining *L. minima* as a legitimate species.

Lemna minima ranges from Minnesota to California, south to Texas and Florida. It also occurs in Mexico, Central America, and South America. In Illinois, it is identified for certain only from three counties.

Specimens Examined: CARROLL: Mississippi Palisades State Park, *Jones 17292* (ILL). MADISON: Swamp east of Granite City, *Evers 31168* (ILLS). WILL: Quarry near mouth of Rock River Brook, *E. J. Hill 183.1906* (F).

8. LEMNA OBSCURA (Austin) Daubs, Ill. Biol. Mon. 34:20. 1965. Map 10. Fig. 7 (k,l,&m).

Lemna minor var. *obscura* Austin in Gray, Man. Bot. 479. 1867.

Fronds obovate to elliptical, 1.5-2.0 mm long, 1.0-1.5 mm wide, obscurely nerved, moderately inflated and cavernous throughout, thin-margined; upper surface flattened to weakly convex, pale green or sometimes slightly reddish, guard cells present, occasionally with a median row of small papules; lower surface moderately to strongly convex, reddish-purple; rootlet one, the sheath cylindrical, reduced, the plants solitary or remaining attached in groups of 2 (-4); rootcap obtuse; fruit obovoid, turbinate; seeds longitudinally ribbed, usually red-pigmented.

This species may not be distinct from *L. minor*, although the data of McClure and Alston (1966) support its recognition. In collections examined from Mason (*R. T. Rexroat 750* and *2222*) and Menard (*R. T. Rexroat 5615*) counties, typical *L. minor* was found mixed with *L. obscura*.

Dried fronds of *L. obscura* are usually pale olive-green and remain inflated, the thin margins frequently becoming involute. A small apical papule may also become prominent.

Lemna obscura ranges in the eastern United States and from Utah to California; it is also reported from the Bahamas and from Mexico. In Illinois, it is found primarily in the central third of the state.

Specimens Examined: CASS: *Rexroat 1290* (ISM). CLAY: Salt Pond N.E. of Clay City, *Ahles s.n.* (ILL). JACKSON: Campbell Lake, *Dixon 5* (SIU). FRANKLIN: Lake W. of Zeigler, *Evers 32594* (ILLS). LAKE: Honey Lake, *C. Standley & R. A. Doubleday 92377* (F). MASON: Four miles N.E. of Saidora, *Rexroat 2222* (ISM). MENARD: S. of Oakford, *Rexroat 5615* (ISM). MORGAN: Waverly Lake, *Carter 14366* (ISM). ST. CLAIR: Seven miles E. of Dupo, *Fuller 13589* (ISM). SANGAMON: Sand Hill, Springfield, *Fuller 2121* (ISM). UNION: Swamp N.E. of Wolf Lake, *Daubs 869* (ILL). STEPHENSON: Lake Le-Aqua-Na State Park, *Stolze 1275* (F).

3. *Wolffiella* Hegelmaier

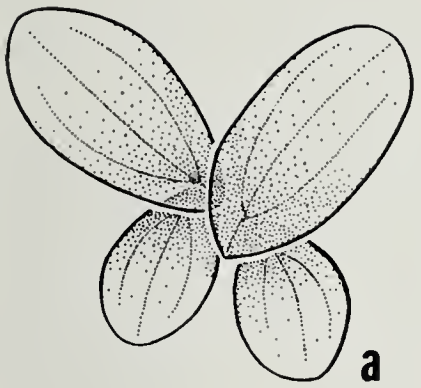
Fronds thin, linear, nerveless; rootlets absent; vegetative reproduction from a single, basal, reproductive pouch, the flowers and fruit rare, produced on the upper surface, ovule one; the young plants remaining attached to form stellate colonies.

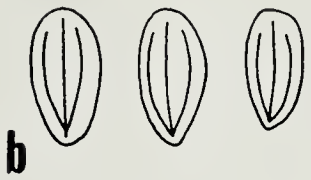
Only the following species occurs in Illinois.

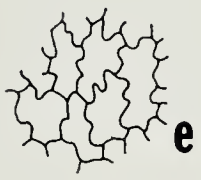
1. WOLFFIELLA FLORIDANA (J. D. Smith) Thompson, Rep. Mo. Bot. Gard. 9:37. 1898. Map 11. Fig. 8 (a, b, & c).

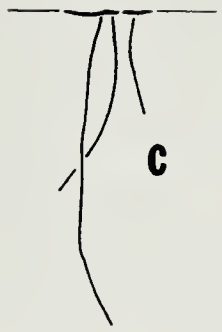
Wolffiella gladiata var. *floridana* J. D. Smith, Bull. Torrey Club 7:64. 1880.

Fronds thin, linear, attenuate, with an acute or obtuse apex, often falcate and curving downward when floating, 3-12 mm long, 0.5-1.0 mm wide, cavernous

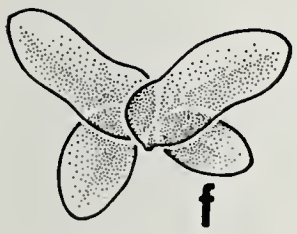


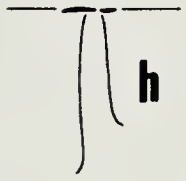




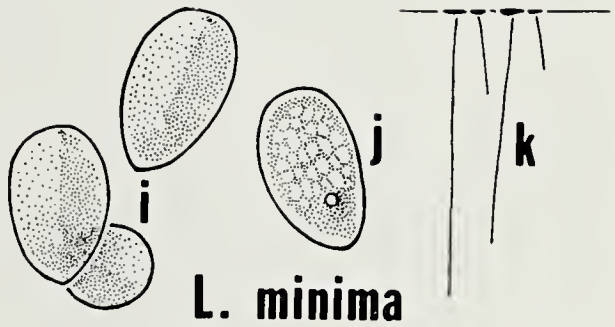


L. trinervis

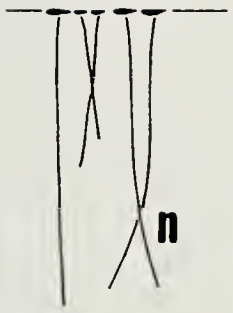




L. valdiviana



L. minima



L. obscura

FIGURE 7.—LEMNA TRINERVIS (Austin) Small, a. habit, dorsal aspect X5; b. morphological variation among mature fronds X2½; c. floating habit, lateral aspect X1; d. winged sheath surrounding rootlet at point of origin on lower surface X25; e. epidermal cell wall pattern, lower surface X200. LEMNA VALDIVIANA Philippi, f. habit, dorsal aspect X5; g. morphological variation among mature fronds X2½; floating habit, lateral aspect X1. LEMNA MINIMA Philippi, i. habit, dorsal aspect X5; j. lower surface of a frond showing inflated central region X5; k. floating habit, lateral aspect X1. LEMNA OBSCURA (Austin) Daubs, l. habit, dorsal aspect X5; m. lower surface of a frond showing dark pigmentation X1; n. floating habit, lateral aspect X1.

throughout, nerveless, guard cells rarely present; plants mostly submerged, solitary or commonly remaining attached in 2-4 or more membered groups, often forming stellate colonies where many members are coherent; new plants arising from a single node situated in a wedge-shaped, often spreading, membranous, basal reproductive pouch; flowers and fruit produced on the dorsal surface but essentially unknown in Illinois specimens.

The strap-shaped fronds and frequently formed stellate colonies readily distinguish this member of the Lemnaceae from any others in Illinois.

Wolffiella floridana is confined to the United States, being known from Massachusetts to Missouri, south to Texas and Florida. In Illinois, it is not common, although it is scattered throughout the state.

Specimens Examined: ALEXANDER: Slough E. of Miller City, *Evers 30427* (ILLS). COOK: Chicago, *Nelson s.n.* (MO). JACKSON: Campbell Lake, *Dixon 5* (SIU). MENARD: Athens, (no collector cited), 1867 (date) (MO). MONROE: Sinkhole N. of Burksville, *Evers 3280* (ILLS). ST. CLAIR: Sinkhole one mile E. of Dupo, *Greenman & Berry s.n.* (MO). UNION: Otter Pond, *Weik 274* (SIU). WABASH: Pond, Mt. Carmel, *Schneck s.n.* (MO).

4. *Wolffia* Horkel

Plants reduced, globoid or ellipsoid, nerveless; upper surface flattened or convex; lower surface strongly convex; pigment cells present in two species; rootlets absent; vegetative reproduction from a single, basal, reproductive pouch; flowers and fruit rarely observed, produced on the upper surface; ovule one.

Key to the Illinois Species of *Wolffia*

1. Plants punctate, flattened above, with a single papule, or with papule absent.
   2. Upper surface with a single accuminate papule, ovate-elliptic .....1. *W. papulifera*
   2. Upper surface flattened, without a papule, elliptic...2. *W. punctata*
1. Plants epunctate, convex above, frequently with a median row of small papules .....3. *W. columbiana*

1. WOLFFIA PAPULIFERA Thompson, Rep. Mo. Bot. Gard. 9:40. 1898. Map 11. Fig. 8 (d,e,f,&g).

Plants obovoid to ellipsoid, 1.0-1.5 mm long, 0.6-1.0 mm wide, nerveless, cavernous throughout, with pigment cells present; upper surface somewhat flattened but gradually rising in the center to form a single, distinct, conical papule, the apex broadly rounded to subacute, guard cells present; lower surface strongly convex; rootlets absent; plants solitary but usually remaining attached on a common axis in groups of 2; flowers and fruit produced on the upper surface, but rarely observed in Illinois specimens.

The single, acuminate papule on the upper surface of this species clearly distinguishes it from the other Wolffias in Illinois.

Wolffia papulifera, as well as *W. punctata*, are extremely difficult to identify in the dried condition. Careful examination under high magnification, however, usually reveals the characteristic surface papule of *W. papulifera*. Both of these plants can be separated from *W. columbiana* on the basis of their pigment cells which remain visible in dried specimens.

Wolffia papulifera is confined to the eastern half of the United States but is also reported from Mexico and Argentina. In Illinois it is rare but scattered throughout the state.

Specimens Examined: ADAMS: Pond, Quincy, *Chase 11664* (ILL). ALEXANDER: Horseshoe Lake, *Daubs 545* (ILL). FRANKLIN: Plumfield Lake, *Fore & Stookey 659* (SIU). HENDERSON: Pond, Oquawka, *Patterson s.n.* (ILL). JACKSON: Campbell Lake, *Dixon 5* (SIU). LAKE: Volo Bog, *Evers 36307* (ILLS). MASSAC: Mermet Lake, *Weik B138* (SIU). MONROE: Sinkhole one mile N. of Burksville, *Hubricht B696* (MO). ST. CLAIR: Sinkhole one mile E. of Falling Spring, *Ownbey s.n.* (MO). UNION: Wolf Lake, *Bailey & Swayne 1890* (SIU). WABASH: Pond, Mt. Carmel, *Schneck s.n.* (ILL).

2. WOLFFIA PUNCTATA Griseb. Fl. BRIT. W. Ind. 512. 1864. Map 13, Fig. 8 (h,i,j,&k).

Plants narrowly ovoid to ellipsoid, 0.6-1.0 mm long, 0.3-0.6 mm wide, nerveless, cavernous throughout with pigment cells abundant; upper surface flattened to slightly convex, the apex subacute to acute, guard cells present; lower surface strongly convex; rootlets absent;

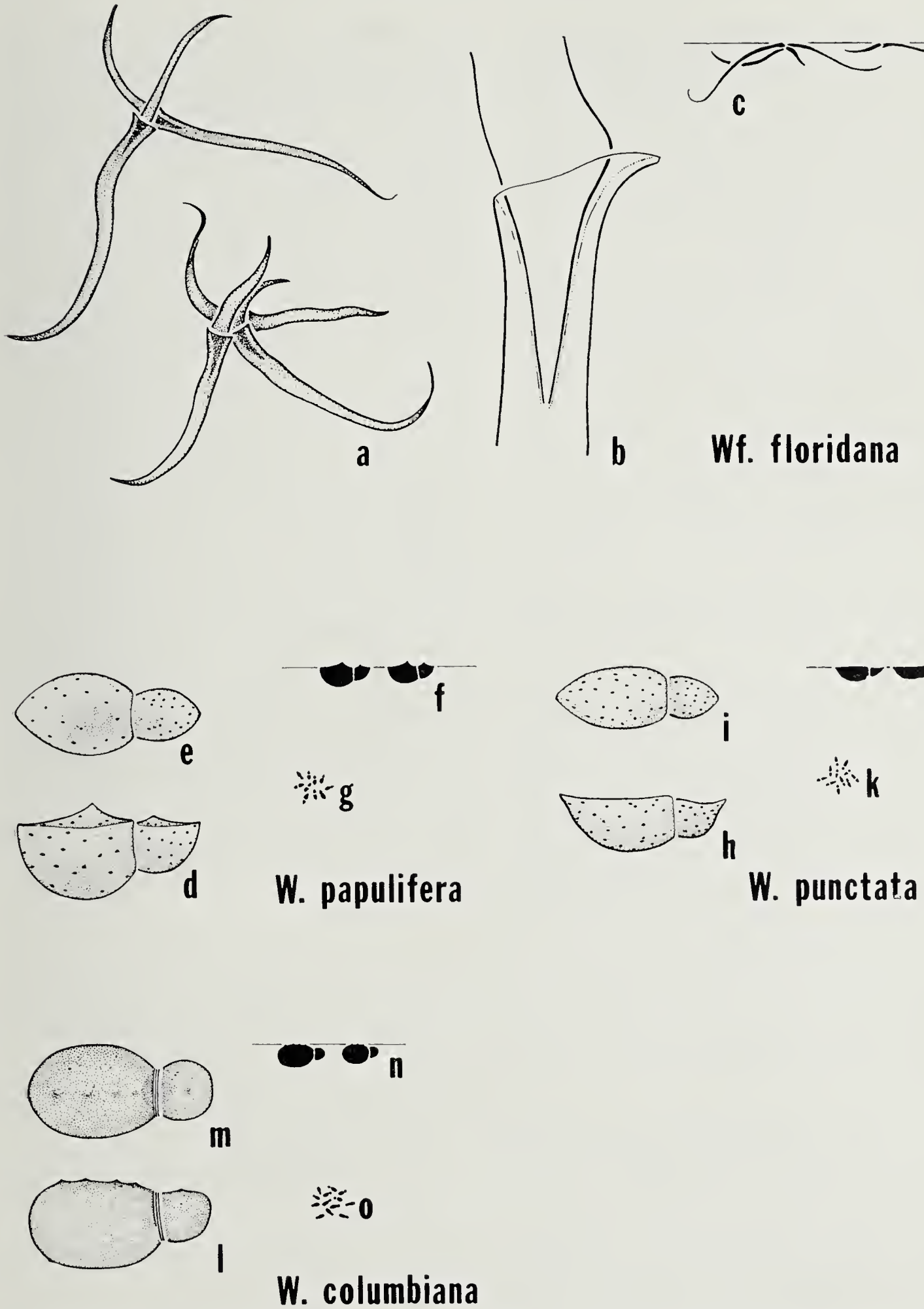


FIGURE 8.—WOLFFIELLA FLORIDANA (J. D. Smith) Thompson, a. habit, dorsal aspect X5; b. basal reproductive pouch showing vegetative origin of new plant X20; c. floating habit, lateral aspect X2½. WOLFFIA PAPULIFERA Thompson, d. habit, lateral aspect X20; e. habit, dorsal aspect X20; f. floating habit, lateral aspect X5; g. floating habit, dorsal aspect X1. WOLFFIA PUNCTATA Grisebach, h. habit, lateral aspect X20; i. habit, dorsal aspect X20; j. floating habit, lateral aspect X5; k. floating habit, dorsal aspect X1. WOLFFIA COLUMBIANA Karsten, l. habit, lateral aspect X20; m. habit, dorsal aspect X20; n. floating habit, lateral aspect X5; o. floating habit, dorsal aspect X1.

plants solitary but usually remaining attached on a common axis in groups of 2; flowers and fruit produced on the upper surface but rarely observed in Illinois specimens.

This species is similar to *W. papulifera*, differing essentially by its lack of the conical, surface papule and by its narrower dimensions.

Like the preceding species, *W. punctata* occurs in the eastern half of the United States. It is also reported from Canada, Mexico, and the West Indies. In Illinois, it is the rarest *Wolffia*, known only from five counties.

Specimens Examined: LAKE: Volo Bog, *Evers 34935* (ILLS). SANGAMON: Sangamon River, *Hall s.n.* (ILL). WABASH: Mt. Carmel, *Schneek s.n.* (ILL). HENDERSON: Oquawka, *Patterson s.n.* (F). MENARD: Athens, *E. Hall s.n.* (F).

3. WOLFFIA COLUMBIANA Karst. Bot Unters. 1:103. 1865. Map 14. Fig. 8 (l,m,n,&o).

Plants globoid to ellipsoid, 0.5-1.2 mm long, 0.5-0.8 mm wide, nerveless, conspicuously cavernous throughout, with pigment cells lacking; upper surface somewhat flattened, often with a median row of minute papules, guard cells present; lower surface broadly convex; rootlets absent; plants solitary but usually remaining attached on a common axis in groups of 2; flowers and fruits arising on the upper surface but rarely observed in Illinois specimens.

Wolffia columbiana, the most widespread species of *Wolffia* in Illinois, differs by its epunctate fronds. Dried specimens become two dimensional but remain easily separated from the other Wolffias.

This species occurs in the eastern half of the United States, in Mexico, Central America, and South America. It is rather common throughout Illinois.

Specimens Examined: ADAMS: Pond, Quincy, *Chase 11664* (ISM). ALEXANDER: Horseshoe Lake, *Evers 32856* (ILLS). CARROLL: *DeFilipps & Ozment 116* (SIU). CHAMPAIGN: Crystal Lake, *Jones 16580* (F). CLAY: Lake Pond N.W. of Clay City, *Ahles 5245* (ILL). COOK: Lake N. of Palatine, *Evers 34838* (ILLS). FRANKLIN: Lake W. of Zeigler, *Evers 32595* (ILLS). FULTON: Rice Lake, *Chase 13316* (ISM). HENDERSON: Ponds, Oquawka, *Patterson s.n.* (ILL). JACKSON: Campbell Lake, *Dixon 5* (SIU). JERSEY: Lake W. of Grafton, *Evers 38868* (ILLS). JO DAVIESS: Mississippi River S. of Blanding, *Evers 35762* (ILLS). KANKAKEE: N.E. of St. Anne, *Fuller 18038* (ILLS). KNOX: Horseshoe Lake, *Weik B137* (SIU). LAKE: Volo Bog, *Fuller 2184* (ISM). McDONOUGH: Three miles N.E. of Macomb, *Myers 1241b* (ISM). MADISON: E. of Granite City, *Evers 31170* (ILLS). MASON: Quiver Lake, *Pepoon & Barrett 9603* (ILLS). MERCER: Slough N. of Keithsburg, *Evers 31942* (ILLS). MONROE: Whiteside Lake, *Shrader s.n.* (ISM). PERRY: Reese Creek E. of Old DuQuoin, *Evers 32713* (ILLS). PUTNAM: Goose Pond, *Pepoon 9602* (ILLS). RANDOLPH: Sinkhole N. of Prairie Du Rocher, *Evers 32783* (ILLS). RICHLAND: Lake one mile N. of Olney, *Weik 156* (SIU). ROCK ISLAND: Slough N. of Milan, *Evers 31906* (ILLS). ST. CLAIR: Sinkhole seven miles E. of Dupo, *Fuller 13590* (ISM). SANGAMON: Sangamon River, *Hall s.n.* (ILL). SHELBY: Pond two & one-half miles N.W. of Windsor, *Fritz s.n.* (ISM). UNION: Wolf Lake, *Bailey & Swayne 1889* (SIU). VERMILION: Pond, Muncie, *Ahles 3428* (ILL). WHITE: Two miles N. of Maunie, *Chapman & Wunderlin 52* (SIU). WHITESIDE: Three miles W. of Prophetstown, *Thompson & Tehon 9606* (ILLS). WILL: *Hill 182.1906* (ILL). WINNEBAGO: Bullhead Slough, Pecatonica River, *Thompson & Tehon 9605* (ILLS).

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Manuscript received January 9, 1968.

ON THE FLIGHT BEHAVIOR OF WORKER *VESPULA MACULIFRONS* (BUYSSON), (VESPIDAE, HYMENOPTERA)

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ABSTRACT. — Counts of worker *Vespula maculifrons* made as they flew from and to their nest hidden in the attic of a cabin at Eaglenest lakes, Minnesota, was the method employed to observe the effects of normal weather conditions on rates of flight. Flights are (1) faster, hence more numerous on bright windless days when temperatures range from 78 to 90° F; but (2) slow at temperatures of 50 to 60°, and prevented at 40°; (3) retarded but not stopped by light to moderate rains under windless conditions; and (4) gradually brought to a stop as light wanes toward nightfall, when stragglers have some difficulty in finding the entrance to the attic but appear to recognize the area from previous exploratory flights. An attempt is made to identify the factors that cause the often pronounced numerical imbalance observed between nestward and outward flights.

On June 17, 1959, a queen of *Vespula maculifrons* was discovered making flights from and to her nesting site in the attic of a one-story cabin. This building stood in the midst of broad-leaved and evergreen trees that shaded it lightly in midday. Temperatures in the attic were moderated somewhat by small screened openings at the ends.

The area of study lies among the Eaglenest lakes near the town of Ely in northeastern Minnesota at latitude about 49 degrees.

METHOD OF STUDY

Since the nest remained invisible and inaccessible within the attic, my means of obtaining data about the colony, as it developed through the summer of 1959, was to count the workers as they passed in and out through a small hole in the siding on the south wall of the cabin. Each of the "counts" extended through 45 consecutive minutes of time, and was also subdivided into 4-minute intervals, as illustrated in Table 1. Thirty-five such counts were made between July 10 and September 18. Twenty of these were selected for presentation in Table 2.

It should be understood that this method does not yield the precise number of workers that constitute the colony at any one time. However, it does show how the rates of flight are affected by the various kinds of weather conditions that prevailed when the count was taken. In general, each worker probably averaged from two to four flights from nest to field and back within the 45-minute periods.

VARIATION IN FLIGHT NUMBERS

Table 1 illustrates a characteristic that is common to all the 20 sample counts (TABLE 2), namely that scarcely ever do even the short 4-minute intervals show identical numbers of departing and returning flights. Certainly there can be no single cause for this continual variation, but it is presumed to arise from combinations of such considerations

as age and experience of the workers, kinds and weights of loads carried, obstacles encountered en route, distances between nest site and sources of food, wood pulp and water, and kinds of weather encountered during the flights. Indications were observed that workers become tired, hence sometimes "take a break" momentarily.

Seasonal Increase in Flights

On June 27, the colony consisted of the mother queen and the first worker of the year. The first count (No. 1, Table 2), taken on July 29, when conditions were nearly optimum for flight, the arriving and departing flights numbered 98 and 97, respectively. It is estimated that 15 workers were then present. By contrast, the last count made on September 18 (No. 20, Table 2) showed that 364 worker flights were made to, and 392 from the nest. The number of workers then present may be estimated from the 67 that were captured by net at the nest site on and soon after September 20. Thus, the large increase in number of flights between July 29 and September 18 is basically traceable to the normal growth of the colony during this period of 7 weeks.

Incidentally, this increase indicates that the queen lived and performed her reproductive functions through a normal lifetime.

However, the fluctuations in number of flights throughout the 20 counts (Table 2) are due to variations in current weather factors, some of which I attempt to identify and evaluate below.

1. *Near-optimum conditions.* Several of the counts recorded in Table 2 point to the combination of weather conditions that are conducive to maximum number of worker flights. These include count numbers 1 and 2, taken on July 29 and August 5, when the worker population still remained relatively small, and temperatures had neared 90°F. just prior to the counts. Arrivals and departures on July 29 numbered 98 and 97, and a week later, on August 5, 158 and 152, respectively. Much of the increase noticed on the latter date, August 5, as compared with July 29, is credited to the emergence of young workers from their cocoons in the meantime.

Table 1.—Illustrates the form used to record in and out flights of workers through a 45-minute period and also 4-minute intervals. At the same time, the table presents the actual counts taken on September 5, 8.20-9.05 PM. This example is summarized at number 11, in Table 2.

Four-minute intervals	Workers arriving	Workers departing	Four-minute intervals	Workers arriving	Workers departing
8.20—8.24	26	18	8.44—8.48	17	6
8.24—8.28	22	12	8.48—8.52	11	6
8.28—8.32	14	16	8.52—8.56	16	3
8.32—8.36	30	13	8.56—9.00	8	0
8.36—8.40	20	12	9.00—9.04	1	0
8.40—8.44	16	12			
			Totals	164	98

More impressive, due to the larger numbers involved, but not more significant as indications of near-optimum conditions for flight, are counts 6, 10 and 12 made on August 21 and September 5 (midafternoon) and 7, when "in" and "out" flights of workers numbered 275-253, 351-321, and 257-238, respectively, in the three instances. Relatively large numbers of flights occurred on these dates, due principally to the larger worker populations then current. However, these maximum numbers of flights were also favored by the temperatures of about 78 and 79°F. and the absence of wind in midafternoons, which were the warmest parts of these days.

2. *Flights in cool weather.* The effects of low summer temperatures on the number of worker flights is well illustrated by the counts taken on the two successive days, September 9 and 10 (Nos. 15 and 16, TABLE 2). These counts contrast sharply with that made on September 11. All these counts were made in midafternoon hours, and the number of workers present in the colony is assumed to have remained fairly constant through the three days.

An electric storm developed at sunrise of September 9 and brought a cold north wind, so that the temperature at count time (3.52-4.37 PM) was still about 56°F. As a consequence, the number of workers arriving at the nest site was held to 69 and the departures to 50.

At sunrise of September 10 (No. 16, TABLE 2), the thermometer registered 39°F.; a 5-minute watch at that time showed no activity at the nest site. But with the north wind subsiding and under a sunny sky, the temperature behind the cabin attained 62° at count time (3.30-4.15), and the number of arrivals increased to 83, the departures to 80.

By contrast, the record of September 11 (No. 17, TABLE 2) serves to show how effectively the higher temperature of 73°F., with bright sun and warming south breeze stimulated the number of worker flights. Under these conditions, arrivals at the nest site totalled 241 and departures 224, in a count taken at 3.02-3.47 PM.

3. *Flight behavior at nightfall.* Retardation in the rate of flight as affected by diminishing natural light is illustrated by two counts taken on September 5. The first one, made at 3.40-4.25 PM at a temperature of about 79°F., showed that the workers moved at the high rate of 351-321 in nestward and woodward flights, respectively in the period of 45 minutes.

Four hours later, at 8.20-9.05 PM, the thermometer reading 75°, and falling, the second count showed 164 worker flights nestward and 98 outward. This extraordinary spread in numbers of inward and outward flights reflects the influence of waning light on movement. However, these final numbers, taken alone, do not describe the manner in which such changes in intensity of light affect the rates of flight.

Table 1 gives particulars of this sample count taken on September 5, 8.20-9.05 PM. First, the counts in this case show both a reduction of total flights and an increased proportion of nestward over outward movements, as compared with the count made in the midafternoon of the same day. That is, at 8.20 PM, the

workers had already b e e n slowed down by waning light and falling evening temperatures. During the 20 minutes between 8.44 and 9.04 PM, the predominance of nestward over outward flights increased at an ever accelerating rate. A lessening number of workers reached the nest site, but the outward flights dwindled at a still faster rate, and ceased, while nestward flights persisted at a low level. The inclination of workers to return to the field was completely inhibited by the darkness prevailing both in and outside of the nest.

The relation of nightfall to flight is exemplified also by a count taken on September 2 (No. 7, TABLE 2). This example confirms the principles demonstrated in the above case.

A word should be added concerning the behavior of stragglers that return to the nest site in darkness. First, while still 10-15 feet away, they seem to be guided vaguely by the dimly visible white wall in which the entrance is located. It is supposed that the worker remembers the site through "photographic images" acquired in previous exploratory flights. As they come closer to the wall, they commonly fly, with an audible thud, against the vertical surface. Failing to hold to the smooth painted wall, they fall earthward, but regain their wings. This falling and flying may be repeated several times, yet the worker seems in general to move toward the entrance. When only a few inches away it somehow recognizes the sur-

TABLE 2.—*Vespula maculifrons*, flights of workers.

Count No's.	Dates of counts	Hours of counts	Temperatures Fahr.	Nestward flights	Outward flights	Weather conditions
1	7.29	3.35— 4.20 PM	90	98	97	near optimum
2	8.5	5.30— 6.15 PM	88	158	152	cloudy, warm, no wind
3	8.6	10.45—11.30 AM	69	76	93	drizzle, north wind
4	8.7	11.40—12.25 AM	62	76	74	cloudy, cool
5	8.8	11.30—12.15 AM	64	97	95	sunny-cloudy
6	8.21	3.15— 4.00 PM	78	275	253	warm, cloudy, no wind
7	9.2	8.30— 9.15 PM	63	84	50	dark at 9.00 PM
8	9.3	11.03—11.48 PM	58	125	81	cool, gusty, light rain
9	9.4	2.26— 3.10 PM	76	358	325	sunny, no wind
10	9.5	3.40— 4.25 PM	79	351	321	warm, no wind
11	9.5	8.20— 9.05 PM	75	164	98	dark at 9.00 PM
12	9.7	2.30— 3.15 PM	78	257	238	sunny, no wind
13	9.8	10.55—11.40 PM	61	109	124	sunny, cool, east wind
14	9.8	2.50— 3.35 PM	72	156	194	sunny, cool, east wind
15	9.9	3.52— 4.37 PM	56	69	50	cold, northwest wind
16	9.10	3.30— 4.15 PM	62	83	80	sunny, cool, 39° F at sunrise
17	9.11	3.02— 3.47 PM	73	241	224	sunny, warm, south wind
18	9.12	3.14— 3.59 PM	68	342	308	sunny, northeast breeze
19	9.13	4.08— 5.03 PM	74	330	320	warming, no wind
20	9.18	4.36— 5.21 PM	70	364	392	sunny, cool breeze, darkening

face runway made familiar through frequent use by itself and others, then, promptly crawls directly into the entrance.

4. *Reactions to rainy weather.* Table 3. The flight behavior of workers during unsettled rainy cloudy weather is reflected in a 45 minute count taken from 11.03-11.48 AM of September 3 (No. 8, TABLE 2). Temperatures varied from 56°F. in the woodland to 63 at the ventilator of the cabin. Moreover, recognizing that weather factors, obtaining previous to the usual count, have conditioned the responses of the workers, I include under period Number 1 (TABLE 3, above), the flights made in 21 minutes. Likewise, I tabulate the flights made in the 24 minutes that follow the regular count, since they show the contrast in number of flights taken between rainy vs. clearing weather conditions.

Deductions. The number of flights made in the first period fall within the expected range, considering the prevailing atmospheric conditions.

The steady brisk rain from dark clouds in the second period, stimulated workers then in the woods to return quickly to the nest site at the rate of 33 in 3 minutes, while it held outward flights to a minimum of 9.

The imbalance between arrivals and departures in the third period stems from the steady but moderate rain. It permitted some outward flights while stimulating a larger number to return to the nest site.

The proportion of nestward to outward flights in the fourth period, when weather tended to clear, indicates that conditions are becoming favorable to woodward movements, after previous confinement to the nest.

The 103 nestward flights made in period 5, after the usual 45 minute count, seem to indicate that many workers delay in the woods during rain of inhibitive proportions, then promptly return to the nest site when rain ceases.

Rainfall, even though not heavy, is more effective than moderate cloud cover alone, as a deterrent to worker flight. The cool air contributed to the comparatively low rate of flights counted.

Imbalance in worker flights. Reference to the counts recorded on Table 2 will show that the number of nestward flights and outward flights are mostly far from equal. On first consideration, it might be supposed that each worker makes a flight back to the nest site for each flight it makes to the surrounding woodland. If this were true, the number of "in" and "out" flights recorded in each of the 45-minute sample periods should ideally be equal. Granted that some degree of error is involved in the counts, and if allowances were made for these errors, there would still remain an imbalance of considerable size. For the sake of discussion, two variations of the imbalance are recognized. In the majority of instances listed in Table 2, the nestward flights more or less outnumber the woodward flights. On the other hand, the outward flights sometimes outnumber those made to the nest.

Search for the explanation of this phenomenon of differences, points to the changing weather conditions, in which the flights take place. Counts numbered 3, 13 and 14 (TABLE 2)

and Table 3 indicate that the predominance of outgoing flights arises from a combination of pre-existing and current conditions either of fair cool weather, or rainy cloudy weather accompanied by lower temperatures. Such conditions become a drag both on outward and nestward flights, and result in an accumulation of workers in the nest. When conditions change subsequently — within hours, to sunshine and warmer, the workers begin moving out, while few return. Somewhat later, a larger number flies away, and more return from the field. Thus is established the numerical superiority of outgoing over nestward flights. This same reaction to a warming trend appears to take place on most mornings of the Eaglenest summer, where the early morning temperatures frequently stand between 40 and 50°F. Assuming that all or most of the worker force passed the night in the nest, there would at first occur a period of outward flight, with few or no returns to the nest in the first minutes. This reaction is followed by a gradual increase in flights as the atmosphere warms toward noon. Assuming that fair weather continues to prevail through the day, the number of flights nestward would, according to counts made (TABLE 2), gradually equal, then exceed, the outgoing flights.

This predominance of flights to the nest is known to occur (TABLE 2) at least from 11.30 to 4.30 PM, in fair weather. Yet it must be supposed that this pattern of flight cannot be maintained so long a time without alternating periods of major outward flights that replenish the woodland with workers for another period of dominant nestward movement. This speculative situation could possibly have been clarified by taking four or more counts per day between early morning and nightfall, instead of the usual single one.

SUMMARY

Counts of worker *Vespula maculifrons* made as they flew from and to their nest hidden in the attic of a cabin at Eaglenest lakes, Minnesota, was the method employed to observe the effects of normal weather conditions on rates of flight. Flights are (1) faster, hence more numerous on bright windless days when temperatures range from 78 to 90°F.; but (2) slow at temperatures of 50 to 60°, and prevented at 40°; (3) retarded but not stopped by light to

TABLE 3.—Observations on Flights with Varying Weather Conditions.

Period number	Periods of observation	Weather conditions during the periods	Flights to the nest	Flights from the nest
1	21 minutes before the 45 minute count	feeble sun, misty, light rain at times	49	66
2	first 3 minutes of the 45 minute count	steady peppery rain, dark, cloudy	33	9
3	next 18 minutes of 45 minute count	rain continued, dark, cloudy	56	30
4	last 24 minutes of 45 minute count	rain slackened, brief sunshine	36	42
5	24 minutes after 45 minute count	no rain, moderately cloudy, more sunshine	103	24

moderate rains under windless conditions; and (4) gradually brought to a stop as light wanes toward nightfall, when stragglers have some difficulty in finding the entrance to the attic but appear to recognize the area from previous exploratory flights. An attempt is made to identify the factors that cause the often pronounced numerical imbalance observed between nestward and outward flights.

Manuscript received February 7, 1968.

STRATIGRAPHY OF THE MIDDLE DEVONIAN SHALES OF SOUTHERN ILLINOIS

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ABSTRACT. — Study of fossils from the Middle Devonian shales of southern Illinois has made possible the differentiation of these beds into two units. The first unit, the Misenheimer Shale, is assigned to the Lingle Limestone as its basal member; the second unit, strata below the Misenheimer and above the Grand Tower Limestone in northern Union County, is tentatively referred to the St. Laurent Limestone.

Rocks of Devonian age were reported from southern Illinois as early as 1857 by Norwood, and formation names were applied to these strata by Savage (1920a). General agreement has not been reached on the recognition of these stratigraphic units, especially in the Middle Devonian above the Grand Tower Limestone. In this paper two exclusively Hamilton-age formations are recognized in southern Illinois, the St. Laurent Limestone (a tentative assignment), and the Lingle Limestone. A rock unit that was originally proposed as a formation, the Misenheimer Shale (Savage, 1920a), is reduced to the status of a member — The Misenheimer Shale Member of the Lingle Limestone (FIGURE 1).

The recognition and reclassification of these strata is the result of detailed faunal studies of Middle Devonian shales in southern Illinois started at Southern Illinois University in 1963 under the direction of Dr. David Nicol, now at the University of Florida. In January, 1965, the study came under the direction of Drs. George H. Fraunfelter and John Utgaard, Southern Illinois University. The author also wishes to acknowledge the aid of Dr. G. Arthur Cooper, U. S. National Museum, Dr. N. Gary Lane, University of California, Los Angeles, and of his fellow students Ira R. Satterfield, John F. Baesemann, and James Norton. Figure 2 was drawn by Mrs. Opal Kurtz.

PALEONTOLOGY AND LITHOLOGY

Paleontology — The Lingle and St. Laurent Limestones consist of alternating limestone and shale beds that reach a maximum thickness of 142 feet in northern Union County (Figures 2 and 3). On the north these beds are truncated by the Rattlesnake Ferry Fault and on the south they are buried by alluvium from the Ohio River. Fossils were collected from the shale beds at nine localities (Table 1), four of which consist of reasonably complete stratigraphic sections and are used as reference sections in this report (Figure 3).

The lowest shale exposed above the *Mierocyelus* zone in sections 3 and 5 contains a distinctive but sparse fauna typified by *Chonetes* sp. and *Nuculites triquetor*, and is here tentatively placed in the St. Laurent Limestone. *Chonetes* sp. and *Nuculites triquetor* do occur

rarely in higher shales, all of which are in the Lingle Limestone, but their relative abundance in the lowest shale bed, plus the absence of species typical of the Lingle shales, distinguishes the lower bed from the shales above.

Shale beds A, B, and C in reference section 1, bed B of sections 3 and 5, and the shale in section 7 all contain a second, higher, fauna typified by *Actinopteria muricata*, *Camarotoechia* sp. and *Productella* sp. This assemblage is characteristic of the Misenheimer Shale Member of the Lingle Limestone. Savage (unpublished manuscript, 1920b) listed the following species from the Misenheimer Shale: *Actinopteria muricata*, *Leiorhynchus limitare* (probably=*Camarotoechia* sp. of this study), *Productella* sp., and *Pterinea* cf. *P. flabella*. The latter species was not found during collection for this report, perhaps because Savage applied the name to specimens of *Actinopteria muricata* with strong radial ornament. The three species cited above as typical of the Misenheimer shale beds at the reference sections are also typical at localities 6, 8, and 9. Other fossils characteristic of the Misenheimer Shale Member are *Ambocoelia* sp., *Rhinocaris* cf. *R. columbina*, *Styliolina fissurella*, and an unidentified fistuliporid bryozoan. Abundant megaspores were also found at localities 7 and 8.

Lithology — In contrast to the faunal differences mentioned above, lithologic distinctions between the shale beds referred to the St. Laurent and shales of the Misenheimer Shale Member are not easily made. At all outcrops the shale in the St. Laurent Limestone is medium to dark brown, thin-bedded, and calcareous on fresh surfaces, and light tan and noncalcareous on weathered surfaces.

Shales in the Misenheimer are variable in their constitution. Generally the Misenheimer is brown to gray shale when unweathered, tan on weathered surfaces, thin-bedded and calcareous in the north, and very thin-bedded to thickly laminated and noncalcareous in the south. The upper shale beds in the Misenheimer in the north more closely resemble the typical Misenheimer of the south than do the lower beds. In the southern part of the area the Misenheimer has been subjected to rather extreme weathering, and its composition is noticeably complicated at locality 6 by mineralization and alteration due to faulting. A few thin limestone beds are present in the Misenheimer to the north (reference sections 1 and 2); these limestones are fossiliferous, but their paleontology and petrology has not been studied.

Discussion of the Stratigraphy

Historical — Savage (1920a, 1920b) believed that in northern Union County and at Grand Tower, Illinois, the Misenheimer Shale was absent, and that the Lingle Limestone, with *Microcyclus*-bearing rocks at its base, rested directly on the Grand Tower Limestone. This interpretation has been reiterated to varying degrees in publications of the Illinois State Geological Survey by J. M. Weller (1940, 1944a, 1944b), Orr (1964), and Meents & Swann (1965), although none of these later authors recognizes the Misenheimer Shale as a valid rock unit. G. A. Cooper *et al.* (1942) and G. A. Cooper (1944) tentatively assigned the *Microcyclus*-bearing rocks in northern Union County and at Grand Tower, Illinois to the St. Laurent Limestone, which

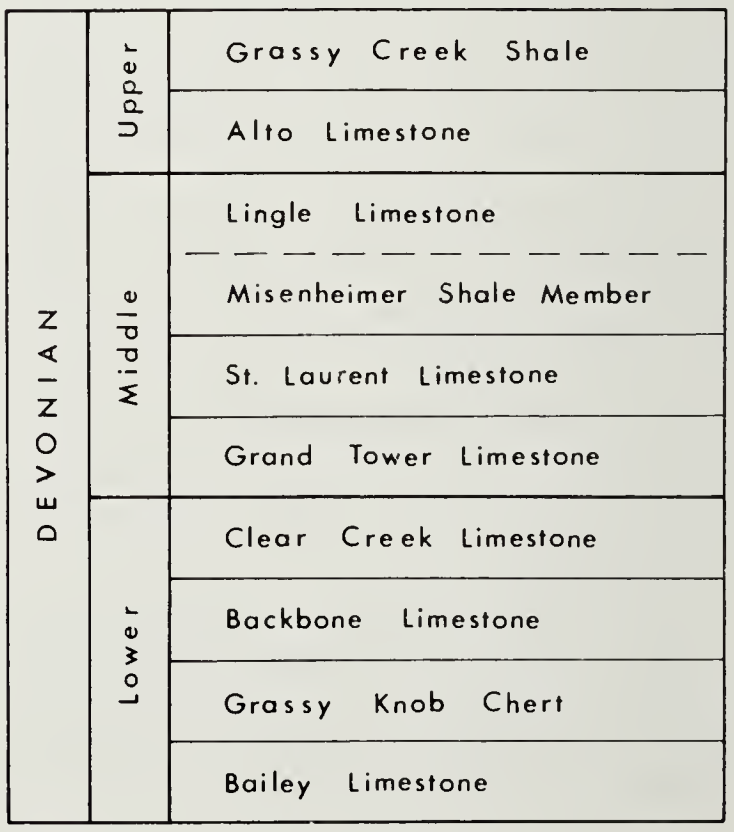


Figure 1. The Devonian formations of southern Illinois.

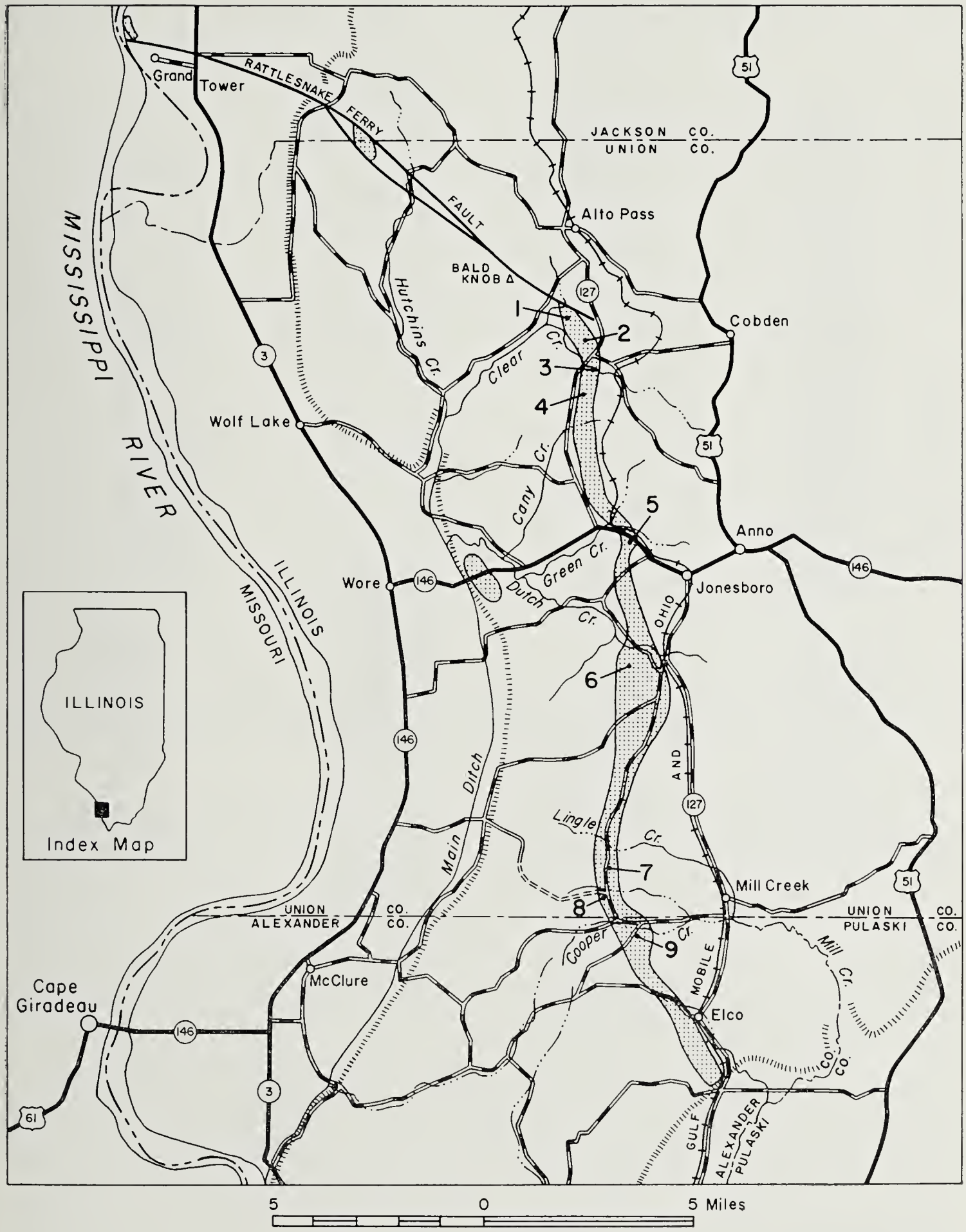


FIGURE 2.—Stipled area indicates outcrop pattern of the combined St. Laurent, Lingle, and Alto Limestones (modified from Weller and Ekblaw, 1940). Large numbers indicate collecting localities. See Appendix for more precise locality data.

had formerly been recognized only in southeastern Missouri. A graphic summary of the various stratigraphic interpretations of the Middle Devonian of southern Illinois is shown in Figure 4.

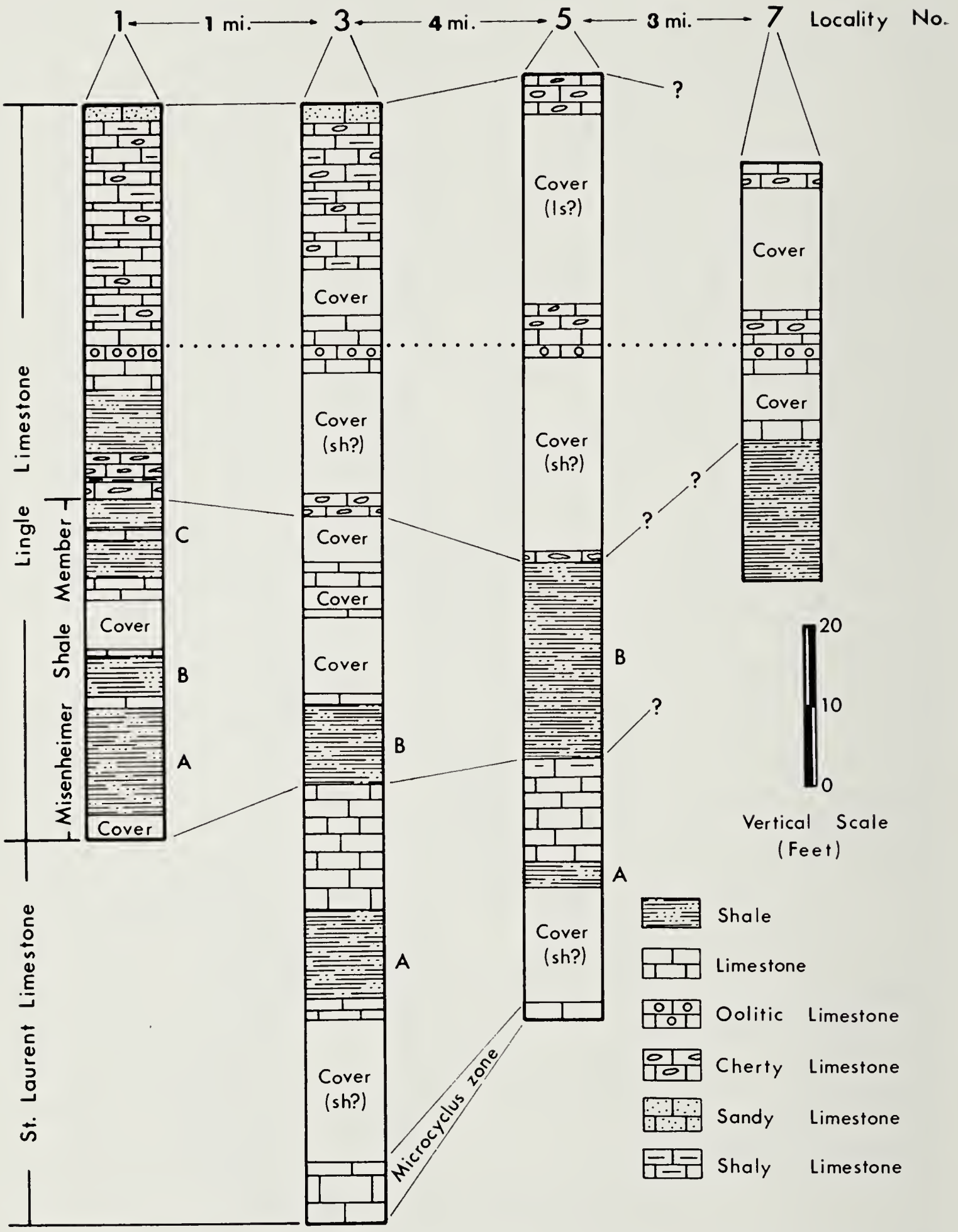
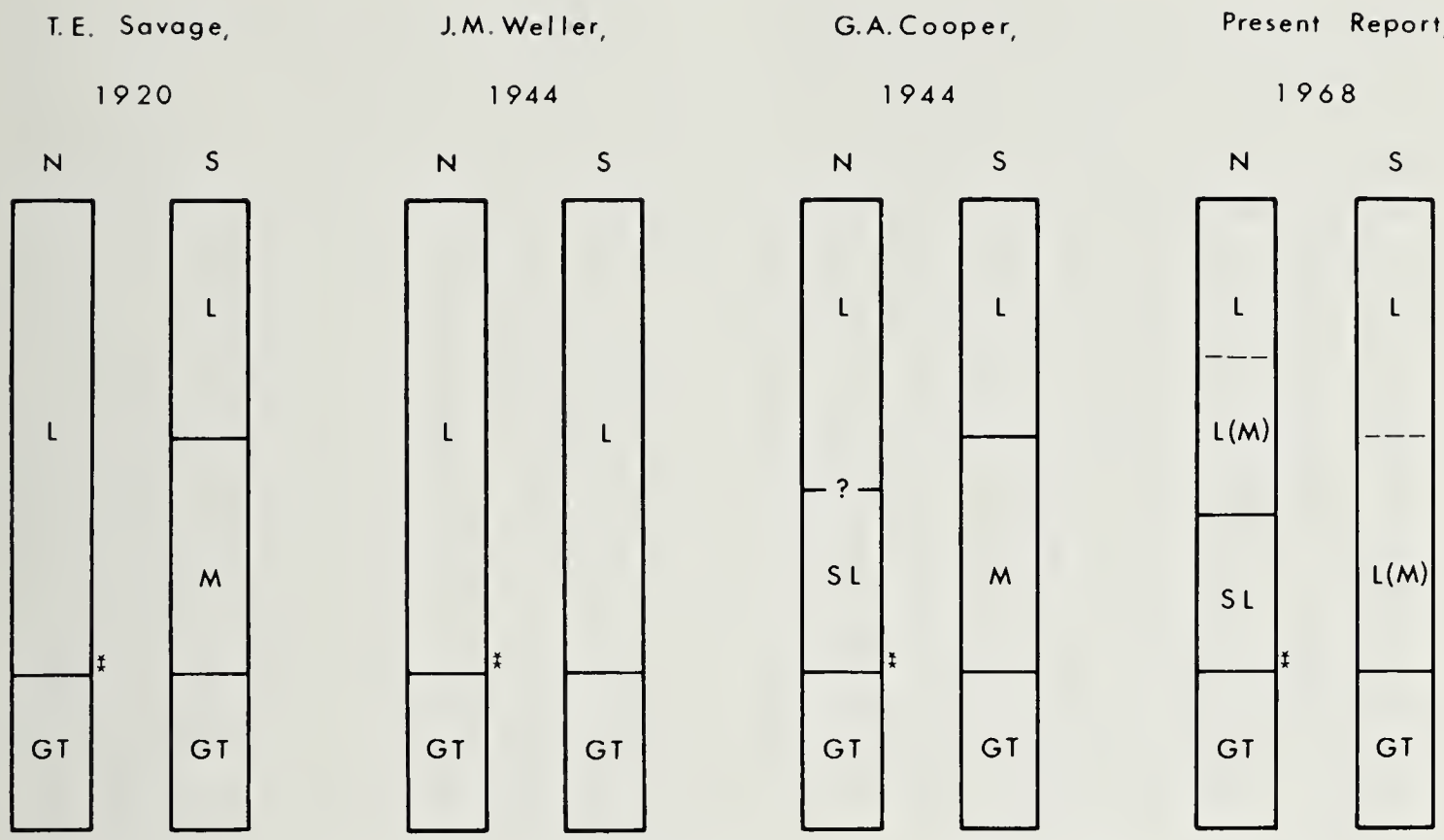


FIGURE 3.—Columnar sections of the Lingle and St. Laurent Limestones. The principal reference section for the Misenheimer Shale Member is at locality 5.

Some of the ambiguities and conflicting interpretations of Middle Devonian stratigraphy of southern Illinois are probably the result of an unfortunate choice of type localities by Savage (1920a) for his Lingle and Misenheimer formations. These localities are in the southern part of the



‡Microcyclus zone, SL—St. Laurent Limestone, GT—Grand Tower Limestone, L—Lingle Limestone, L(M)—Lingle Limestone, Misenheimer Shale Member, M—Misenheimer Shale

FIGURE 4.—Summary of interpretations of Middle Devonian stratigraphy in southern Illinois. Each author's interpretation of the stratigraphy in the northern (N) and southern (S) parts of the outcrop area is represented. Not to scale.

outcrop belt and although the sequence is thicker and the exposure is better in the north, Savage apparently felt compelled to designate the type areas where he did because of the close relationship between his two formations and the supposed absence of the Misenheimer in the north.

The Misenheimer Shale Member—J. M. Weller (1944a) recommended suppression of the name Misenheimer on the basis that the unit could not be recognized consistently, and included all strata from the top of the Grand Tower Limestone to the base of the Alto Limestone in the Lingle. G. A. Cooper (1944) disagreed with Weller's definition of the Lingle, but in his short discussion of the Misenheimer did not define the stratigraphic or geographic boundaries of the unit.

The present reappraisal of the status of the Misenheimer stems from recognition of the Misenheimer fauna in northern Union County in shales 56 and 34 feet above the Grand Tower at localities 3 and 5 respectively, and the evidence for lateral continuity of these beds with Savage's type Misenheimer furnished by fossil collections from nine localities and extensive field mapping (especially by Ira R. Satterfield). This evidence indicates that a more refined stratigraphic interpretation can now be made and that such an interpretation must include the Misenheimer.

There are two reasons for assigning these shales to the status of the Misenheimer Shale Member of the Lingle Limestone. The Middle Devonian beds above the St. Laurent

Limestone form a coherent sequence. The close relationship of the Misenheimer to the Lingle is especially apparent in the northern part of Union County where there are several thin limestone beds that interfinger with the shales (FIGURE 3). This close stratigraphic relationship is the principal reason for the inclusion of the Misenheimer in the Lingle Limestone. Also, considering the typically poor exposure it is not generally practicable to map such a thin shale unit. Although this last point is subjective, the author feels that it lends considerable weight to the argument for the inclusion of the Misenheimer in the Lingle.

Under the code established by the American Commission on Stratigraphic Nomenclature (1961, Article 13, Remark (*h*)) type sections cannot be moved; however, Article 13, Remarks (*a*) and (*i*) permit the establishment of reference sections and a principal reference section when for various reasons the type section is inadequate. The type locality established for the Misenheimer by Savage (1920a, 1920b) is locality 8 of this report. At this locality neither the upper nor lower contact is well exposed, although the Misenheimer does appear to be resting on the Dutch Creek Sandstone which is the basal member of the Grand Tower

TABLE 1.—Distribution and abundance of fossils in the Middle Devonian shales of southern Illinois.

Fossils	Locality												
	1A	1B	1C	2*	3A*	3B	4*	5A*	5B	6	7	8	9
Rhinocaris cf. *R. columbina*	. . . .	R	. . . .	. . . .	. . . .	C	. . . .	. . . .	R	R	. . . .	. . . .	. . . .
Lingula sp.	. . . .	. . . .	. . . .	. . . .	R	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Orbiculoidea sp.	. . . .	. . . .	. . . .	. . . .	. . . .	C	. . . .	. . . .	R	. . . .	. . . .	. . . .	. . . .
Ambocoelia sp.	C	A	. . . .	. . . .	. . . .	A	. . . .	. . . .	R	C	. . . .	. . . .	. . . .
Camarotoechia sp.	. . . .	C	. . . .	R?	. . . .	R	C?	. . . .	A	A	A	A	C?
Chonetes sp.	. . . .	. . . .	. . . .	. . . .	R	. . . .	A	C	. . . .	. . . .	C?	R?	. . . .
Cupulorostrum? sp.	. . . .	R	. . . .	. . . .	. . . .	A	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Productella sp.	. . . .	. . . .	R	. . . .	. . . .	A	. . . .	. . . .	A	C	R	R	R
Michelinoceras sp.	. . . .	. . . .	. . . .	. . . .	. . . .	R	R	. . . .	C	. . . .	. . . .	. . . .	. . . .
Spyroceras sp. A	R?	. . . .	R?	. . . .	R?	. . . .	C	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Spyroceras sp. B	. . . .	. . . .	. . . .	. . . .	. . . .	R	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Spyroceras sp. C	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	R	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Tornoceras (*Tornoceras*) cf. *T.* (*T.*) *uniangulare*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	R	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Actinopteria muricata	R	C	R	. . . .	. . . .	A	. . . .	. . . .	A	A	C	C	. . . .
Cornellites sp.	. . . .	. . . .	. . . .	. . . .	. . . .	R	. . . .	. . . .	R	. . . .	. . . .	. . . .	. . . .
Leiopteria cf. *L. leavis*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	R	R	. . . .	. . . .	. . . .
Leptodesma sp.	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	R	. . . .	. . . .
Modiella? sp.	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	R	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Modiomorpha sp.	. . . .	. . . .	. . . .	. . . .	R	. . . .	C	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
Nuculites triquetor	. . . .	. . . .	R	R	R	R	A	C	. . . .	. . . .	. . . .	. . . .	. . . .
Phthonia cf. *P. cylindrica*	. . . .	. . . .	. . . .	. . . .	R	. . . .	C	. . . .	R	. . . .	. . . .	. . . .	. . . .
Fistuliporid Bryozoan	R	C	R	. . . .	. . . .	A	. . . .	. . . .	A	R	. . . .	. . . .	. . . .

*=St. Laurent horizon, all other locality numbers refer to shales in the Lingle.
?=Identity in question

R = Rare (1 - 3 specimens)
C = Common (4 - 12 ")
A = Abundant (Over 12 ")

formation. Because of the inadequacy of the type locality it is proposed that the sections at localities 1, 3, 5, and 7 illustrated in Figure 3 be recognized as reference sections, and that of these locality 5 be recognized as the principal reference section.

Where exposed the upper contact of the Misenheimer is sharply defined, but the lateral persistence of thin black cherty limestone above, and of other thin limestone beds in the Lingle suggests that the entire sequence is conformable. At locality 5 the lower contact of the Misenheimer is gradational; the upper few inches of the massive limestone bed at the top of the St. Laurent become argillaceous and thin-bedded. Farther south the Misenheimer rests on the basal Dutch Creek Sandstone Member of the Grand Tower Limestone with what is probably a local unconformity.

There is a general decrease in the proportion of carbonates as one goes south along the Devonian outcrop in southern Illinois and it is not entirely clear whether this is the result of a facies change, or carbonate solution by weathering, or both. J. M. Weller (1944a) discussed the case for weathering and concluded that for the most part the decrease in carbonates was due to that cause. Later, Meents and Swann (1965) tried to resolve some of the stratigraphic problems encountered in the Grand Tower by proposing a structural feature they named the Wittenburg Trough, which was active in the Devonian and extended from southern Illinois northwest into Missouri following the boundary between the Ozarks and the Illinois Basin.

Strata of the St. Laurent and upper Grand Tower formations were not seen south of Jonesboro, Illinois, and the Misenheimer appears to rest on the Dutch Creek Sandstone in southern Union County. Some limestone blocks from the Lingle are slumped over the Misenheimer at locality 8, but Lingle carbonates are not seen farther south. It is this author's opinion that thinning of the Middle Devonian section and gradual disappearance of carbonates to the south indicates that the rocks to the south were deposited shoreward relative to the rocks farther north, probably near the edge of the Wittenburg Trough.

Conclusions

1. The status of the Misenheimer Shale is reevaluated and it is designated here the Misenheimer Shale Member of the Lingle Limestone.

2. The Misenheimer Shale Member is traced the full length of the Middle Devonian outcrop in Union and Alexander Counties, southern Illinois.

3. Strata below the Misenheimer Shale Members of the Lingle Limestone and above the Grand Tower Limestone are tentatively referred to the St. Laurent Limestone as suggested by Cooper (1944).

4. A much larger fauna is reported from the Middle Devonian shales of southern Illinois than was previously known.

Appendix of Localities

All collecting localities are listed below. The sequence of numbering agrees with that used on all figures and tables. When the locality number is followed by a letter e.g. 3B, the letter denotes a particular shale unit as illustrated in figure 3.

1. SE¼ SW¼ sec. 27, T. 11 S., R. 2 W., 3 Principal Meridian, 3½ miles west of the Village of Cobden and 2 miles south of the Village of Alto Pass, Union County, Illinois. This is the most northerly good exposure of the Lingle Limestone. It is ¼ mile north of the sharp bend in the county road in the NW¼ sec. 27, same township. See figure 3 for a columnar section of the Lingle Limestone at this locality.

2. NW¼ SE¼ sec. 27, T. 11 S., R. 2 E., 3 P.M.; 3¼ miles west of the Village of Cobden, and 2½ miles south of the Village of Alto Pass, Union County, Illinois. This is a small exposure of the lowest shale unit. It is on the east hillside of a field adjacent to the gravel road in sec. 27, same township.

3. NW¼ NE¼ sec. 34, T. 11 S., R. 2 W., 3 P.M.; 3¼ miles west of the Village of Cobden, and 3¼ miles south of the Village of Alto Pass, Union County, Illinois. *Microcyclus*-bearing rocks crop out in the south bank of Clear Creek 200 feet east of the bridge on Illinois State Highway 127. It is extremely difficult to accurately measure the stratigraphic section at this locality. See figure 3 for a columnar section.

4. NW¼ SE¼ sec. 34, T. 11 S., R. 2 W., 3 P.M.; 3¼ miles west of the Village of Cobden, and 3¾ miles south of the Village of Alto Pass, Union County, Illinois. This is a very small exposure in the base of the main drainage from Iron Mountain. The shale here is very calcareous and contains a distinctive assemblage of fossils.

5. NE¼ NW¼ sec. 23, T. 12 S., R. 2 W., 3 P.M.; 1¾ miles NW of the Town of Jonesboro, Union County, Illinois. *Microcyclus*-bearing rocks crop out in the south bank of Green Creek 300 feet SW of the bridge across Illinois State Highway 146. There has been much slumping in the upper part of the section at this locality. See figure 3 for a columnar section.

6. SW¼ NW¼ sec. 2, T. 13 S., R. 2 W., 3 P.M.; 3 miles SW of the Town of Jonesboro, Union County, Illinois. Access to this locality is best gained by using the trail which goes south from the main road just west of the center of sec. 35, T. 12 S., R. 2 E., 3 P.M. Approximately 20 feet of shale is exposed in the east bank of the main drainage. The shale here closely resembles the "calico rock" found in the Springville Shale. The colors vary from white to tan with maroon patches and streaks. A fault is exposed at the north edge of the outcrop.

7. SW¼ SW¼ sec. 26, T. 13 S., R. 2 W., 3 P.M.; 3 miles WNW of the Village of Mill Creek, Union County, Illinois. The outcrop is immediately adjacent to the main county road in the SW¼ sec. 26, at the concrete ford on Lingle Creek. This exposure is the type section of the Lingle Limestone. See figure 3 for a columnar section.

8. SW¼ NW¼ sec. 35, T. 13 S., R. 2 W., 3 P.M.; 3 miles west of the Village of Mill Creek, Union County, Illinois. The exposure is in the south bank of a branch of Cooper Creek about 400 feet west of the main county road in sec. 35; and is the type section of the Misenheimer Shale.

9. NE¼ SE¼ sec. 2, T. 13 S., R 2 W., 3 P.M.; 2½ miles WSW of the Village of Mill Creek, Alexander County, Illinois. The exposure is in the creek bed 150 feet south of the farmhouse at the above location. This is the southern-most exposure of Lingle Limestone and the only exposure observed in Alexander County.

Acknowledgments

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Manuscript received January 8, 1968.

CHLOROPHYLL *a* IN THE ILLINOIS RIVER

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ABSTRACT. — Chlorophyll *a* in the Illinois River near Peoria was measured during the summer and fall of 1966. Highest chlorophyll *a* concentration observed was 0.496 mg/liter on 7 July 1966.

The effect of enrichment on the biota of the Illinois River by effluent from industrial and domestic wastes is well documented (Mills, Starrett and Bellrose, 1966). In 1963, it was estimated that a BOD population equivalent of 1,170,000 was being discharged into the river or its tributaries above Peoria. As a result, phosphorus concentrations at Peoria were observed to be among the highest of 128 sampling stations (Public Health Service Water Pollution Surveillance System, 1963). A comparative study of 103 stations on the major rivers and Great Lakes of the United States by Williams (1964) revealed that the Illinois River at Peoria exhibited low phytoplankton diversity coupled with extremely high phytoplankton counts.

Chlorophyll *a* concentration in the Illinois River was determined during the summer and fall of 1966. The concentration of this substance was measured to determine the effect of organic enrichment on the phytoplankton and to establish a base line for further studies. Organic enrichment of the Illinois River should allow support of large standing crops of phytoplankton. As a result, chlorophyll *a* concentration should be higher than that in a river not receiving organic effluent. In effect, chlorophyll *a* is being used as an indicator of organic enrichment. The term chlorophyll *a* is equivalent to chlorophyll "A" as used by Odum, McConnell and Abbott (1958) and refers to . . . . "an extractable pigment fraction defined spectrophotometrically. It is recognized that more than one chemical entity may be involved."

PROCEDURES

Ten stations along an eight mile reach of the Illinois River near Peoria were selected for study. Stations were numbered by miles upstream from the Mississippi River (FIG. 1). The upper station was located in the lower end of upper Peoria Lake while the lower station was located below the city. Water depth at all stations was relatively shallow and ranged from two to four meters during the study.

Water samples were taken from the surface approximately once each week from each station between 17 June 1966 and 30 November 1966. Aliquots of 100 ml were filtered through membrane filters of 0.45 micron pore size for chlorophyll analysis. The residues, consisting of algal cells and other particles, were extracted in 10 ml of 90% acetone and refrigerated in the dark at approximately 5C for 24 hours. Upon removal, the samples were centrifuged and optical density of the liquid was determined with a Bausch and Lomb Spectronic 20 photoelectric colorimeter with a 2.54 cm cuvette at 665 millimicrons.

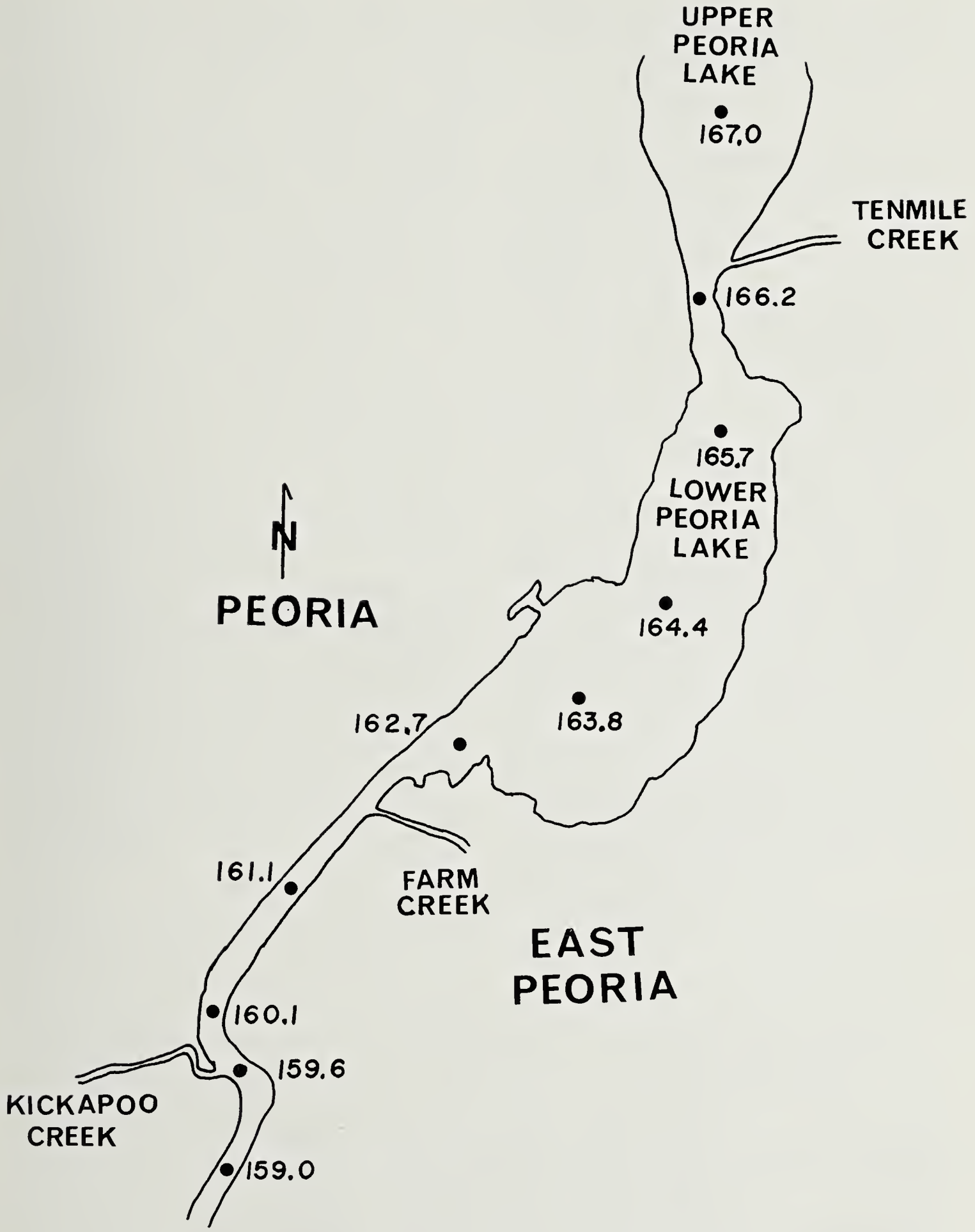

FIGURE 1.—Map of Illinois River near Peoria. Station locations indicated by numerals.

The methods of Odum et al. (1958) were used to estimate chlorophyll *a* concentration where chlorophyll *a* in mg/liter of 90% acetone = 13.4 d_{665}. After correction for acetone and sample volumes this equation became:

Chlorophyll *a* in mg/liter = 1.34 d_{665}

Water temperature and dissolved oxygen were measured in the field according to standard methods. Turbidity was determined in the laboratory by the use of a Bausch and Lomb photoelectric

colorimeter with a 2.54 cm cuvette at d_{450}. The pH was measured by means of a portable Sargent pH meter.

Results and Discussion

Dominant photosynthesizing organisms collected during this study were *Melosira* sp., *Stephanodiscus* sp., *Nitschia* sp., *Cyclotella* sp., *Ankistrodesmus* sp. and *Scenedesmus* sp. The highest chlorophyll *a* concentration observed during the study was 0.496 mg/liter at Station 166.2 on 7 July 1966 (Table 1). Chlorophyll concentration was highest during the summer months when sunlight and water temperatures were maximum. Chlorophyll concentration in oil refinery effluent holding ponds was also greatest during late spring and summer when the water was warmer and sunlight more intense (Copeland, Minter and Dorris, 1964).

Chlorophyll concentration is also apparently dependent upon the amount of available nutrients (Odum *et al.*, 1958). Bacterial degradation of organic materials in the Illinois River could proceed more rapidly during warmer months and thus release nutrients to the photosynthesizing organisms at a faster rate than during colder months. Copeland *et al.* (1964) reported maximum chlorophyll in an oil refinery effluent holding pond located nearest the effluent source during late June and early July. They concluded that large amounts of nutrients were available earlier in the pond system at higher temperatures.

The same combination of environmental factors encountered in the study of oil refinery effluent holding ponds was undoubtedly responsible for higher chlorophyll readings in the Illinois River during summer. In addition, however, turbidity was considerably lower during summer than fall (Table 2). Turbidity refers to the degree of opaqueness produced in water by suspended particulate matter (Reid, 1961). In the Illinois River,

Table 1.—Chlorophyll *a* Concentration in the Illinois River

Station	Summer*		Fall**	
	Range (mg/l)	Mean ± SD (mg/l)	Range (mg/l)	Mean ± SD (mg/l)
167.0	0.035—0.335	0.192±0.097	0.065—0.174	0.132±0.034
166.2	0.052—0.496	0.204±0.147	0.065—0.174	0.138±0.034
165.7	0.037—0.335	0.133±0.085	0.065—0.164	0.122±0.032
164.4	0.060—0.295	0.134±0.064	0.070—0.189	0.127±0.039
163.8	0.080—0.288	0.149±0.057	0.065—0.150	0.124±0.026
162.7	0.042—0.348	0.172±0.075	0.065—0.217	0.140±0.042
161.1	0.052—0.268	0.137±0.060	0.065—0.201	0.120±0.051
160.1	0.040—0.335	0.188±0.094	0.070—0.174	0.131±0.034
159.6	0.038—0.415	0.167±0.093	0.070—0.261	0.145±0.060
159.0	0.032—0.375	0.150±0.107	0.070—0.173	0.121±0.033

* Data based on 10 samples per station.
** Data based on 8 samples per station except for Station 167.0. This Station was sampled 7 times.

TABLE 2.—Mean Physico-Chemical Conditions

Station	Temperature C		Oxygen (ppm)		Turbidity (ppm)		pH	
	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall
167.0.....	27.5	12.4	6.5	8.6	45	76	7.8	7.5
166.2.....	27.1	12.4	6.3	8.3	55	86	7.8	7.6
165.7.....	26.7	12.4	6.1	8.3	47	73	7.9	7.8
164.4.....	26.7	12.7	5.8	8.5	46	71	7.7	7.9
163.8.....	26.8	12.7	5.7	8.7	47	72	7.9	7.7
162.7.....	27.0	12.5	7.3	8.5	47	72	7.9	7.9
161.1.....	26.9	13.4	5.4	7.8	52	71	7.8	7.8
160.1.....	26.8	13.1	4.6	8.2	53	74	7.8	7.8
159.6.....	26.6	13.1	4.6	8.1	50	85	7.6	7.6
159.0.....	27.2	13.1	4.6	8.4	48	76	7.7	7.7

this condition is attributed primarily to a heavy silt load.

Effluents entering the Illinois River at Peoria had little immediate effect on chlorophyll concentration. Bacterial degradation apparently does not proceed rapidly enough to provide large quantities of additional nutrients for the phytoplankton in the immediate area. Evidence that some degradation of organic materials occurred as the water mass passed Peoria is shown by the decrease in dissolved oxygen at downstream stations during summer (TABLE 2). During fall, however, degradation apparently proceeded at a much slower rate since all stations exhibited approximately the same dissolved oxygen concentrations. Nutrients added to the river at Peoria and Pekin are moved downstream to the La Grange pool and provide even greater enrichment. As a result, dissolved oxygen in this pool is extremely low (Mills *et al.*, 1966).

Chlorophyll concentrations in various phytoplankton communities varies throughout the world but there is less of the pigment in unpolluted waters (Copeland *et al.*, 1964) than in the Illinois River. Bartsch and Allen (1957) reported a maximum concentration of 7.32 mg/1 of chlorophyll *a* in one sewage pond during summer. This concentration is considerably greater than the summer maximum in the Illinois River. In this same study, however, chlorophyll *a* concentrations in other sewage ponds ranged from 0.0004 mg/1 to 0.65 mg/1, quantities not too unlike the range (0.032 mg/1 to 0.496 mg/1) in the Illinois River during summer.

In a study of chlorophyll *a* in oil refinery effluent holding ponds, Copeland *et al.* (1964) reported a maximum concentration of 1.350 mg/1 during summer, a concentration approximately three times that found in the Illinois River.

Sewage ponds and oil refinery effluent holding ponds contain small volumes of water in comparison to the volume of water in the Illinois River, yet all three systems exhibit high concentrations of chlorophyll *a*. This condition is to be expected in sewage

ponds and oil refinery effluent holding ponds where highly concentrated nutrients are continuously being added. Two factors are apparently responsible for the high chlorophyll *a* concentration in the Illinois River. First, the high calcium hardness of the water apparently helps support large standing crops of phytoplankton (Williams, 1966). The second and most important reason is the enrichment caused by the presence of untreated or partially treated sewage and industrial wastes from municipalities and industries situated near the river or its tributaries.

Acknowledgment

This study was supported by a Bradley University Faculty Research Grant.

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Manuscript received April 3, 1968.

POLLEN PORE NUMBER AND POLYPLOIDY IN *RUBUS*

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ABSTRACT. — The pollen from several cultivars and selections of *Rubus* was examined and classified according to pore number. A range from monotreme to hexatreme was found among the diploid, tetraploid, and hexaploid forms examined. The tritreme class was predominant in all of the diploids, readily separating this group from the tetraploids and one hexaploid which were all characterized by a predominance of the tetratreme class. Pollen pore number alone was not sufficient to separate the hexaploid from the tetraploids.

It has been found, in more closely studied genera than *Rubus*, that differences in pollen morphology, particularly in the number of pores, were related to varying numbers of somatic chromosomes. In the genus *Oenothera*, Lutz (1917) reported mostly tritreme pollen for diploid forms, a mixture of tritreme (predominant type) with tetratreme pollen for triploid forms, and characteristically tetratreme with relatively few tritreme pollen grains for tetraploid forms. Zander (1938) confirmed this pore number-chromosome number relationship between a diploid and tetraploid form of *Oenothera* and showed a similar relationship in *Fuchsia*. Maurizio (1956) examined pollen from diploid and polyploid forms of *Datura, Lobelia, Monarda, Nicotiana, Salvia,* and *Trifolium* and concluded that in all of the species examined the multiplication of the chromosome number led to an increase in the number of germinal apertures in the pollen grains. More recently, Laws (1965) showed an increase in pollen pore number with increasing polyploidy in diploid, tetraploid, and octoploid plants of *Oenothera* and related an increase in range of pore number to a decrease in regularity of chromosome distribution during meiosis.

Published information on *Rubus* pollen morphology seems extremely limited, confined largely to descriptive summaries of *R. chamæmorus* pollen (Erdtman, 1943, 1966; Erdtman, *et al.*, 1961), of *R. idæus* pollen (Erdtman, *et al.*, 1961; Maurizio and Louveaux, 1965) and of *R. fruticosus* pollen (Maurizio and Louveaux, 1965). In relation to pore number, the pollen of these three species is classified as tritreme, the general rosaceous type, without reference to somatic chromosome number. Fischer, *et al.*, (1943) reported that pollen and seed size of tetraploid *Rubus* was consistently larger than that of the diploids with which they were compared, with no reference to pore number.

The purpose of this work was to examine the possibility of estimating somatic chromosome numbers in *Rubus* through correlation with pollen pore number.

Materials and Methods

The pollen used in this study was obtained from plants of known ploidy grown at the Small Fruit Research Center, Southern Illinois University, Carbondale, Illinois and at the Pomology Research Center, University of Illinois, Urbana, Illinois. The flower buds were collected into gelatin capsules at a stage just preceding anthesis and stored in a dessicator over calcium chloride at 32-34° F until preparation for microscopic examination. Whole mounts of the stored pollen for observation of pore numbers were prepared according to the method described by Conn, *et al*, (1960) using methyl green stain in glycerin jelly.

The optical system used in taking the photographs was a Zeiss photomicroscope with either a Planapochromat 100/1.32 or Apochromat 40/1.0 oil immersion objective.

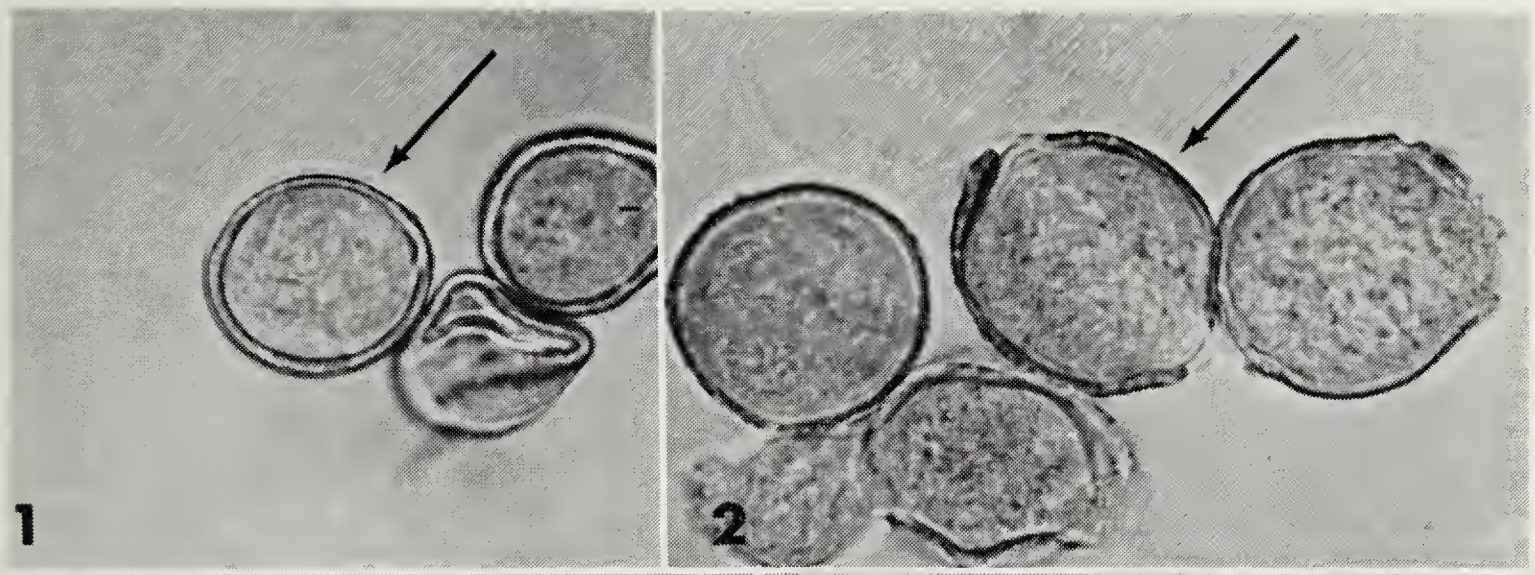
Results and Discussion

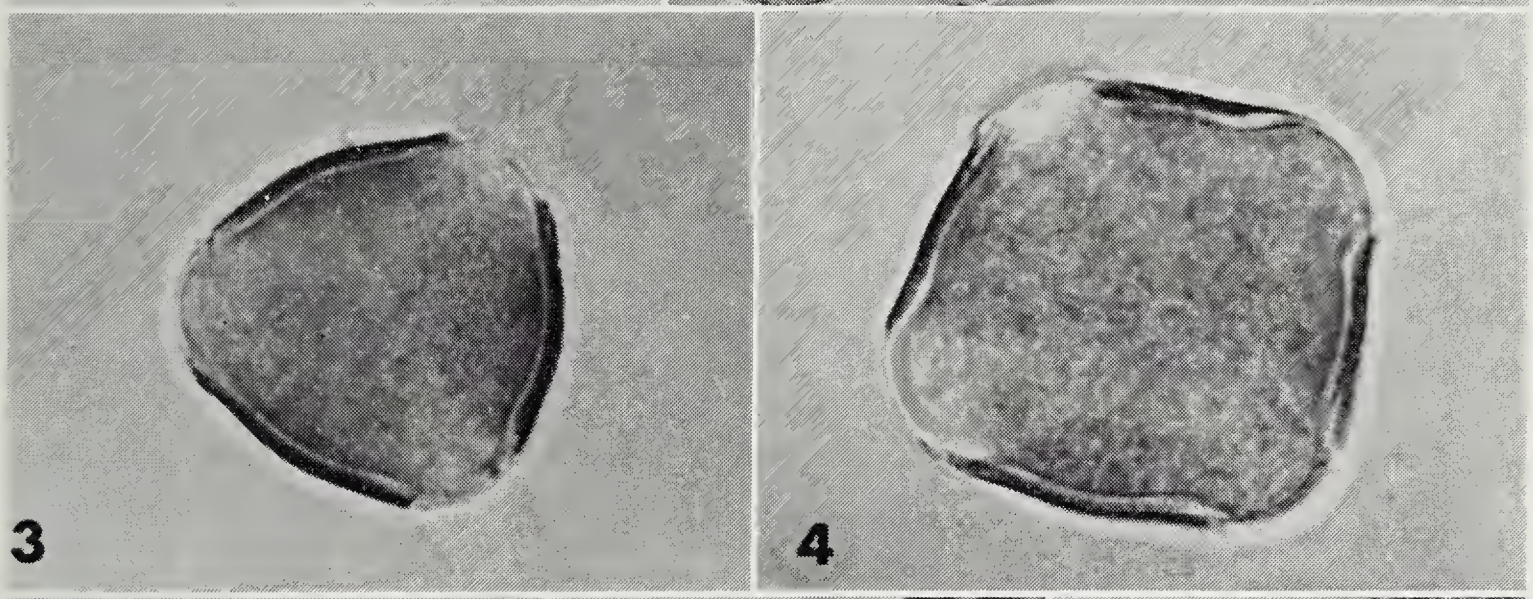
Classification of the pollen according to pore number from red, black, and purple raspberries and an erect blackberry cultivar ranged from monotreme to hexatreme as illustrated in Figures 1-6b and summarized in Table 1. The finding that even some diploid forms may produce hexatreme pollen imposes a requirement for scoring more than a few pollen grains per sample to avoid error in estimating somatic chromosome number. In the sample of 'Blackhawk' pollen, for example, only one of 73 pollen grains scored was hexatreme, but this single exception eliminates the use of this pore class as a means of separating polyploids from diploids.

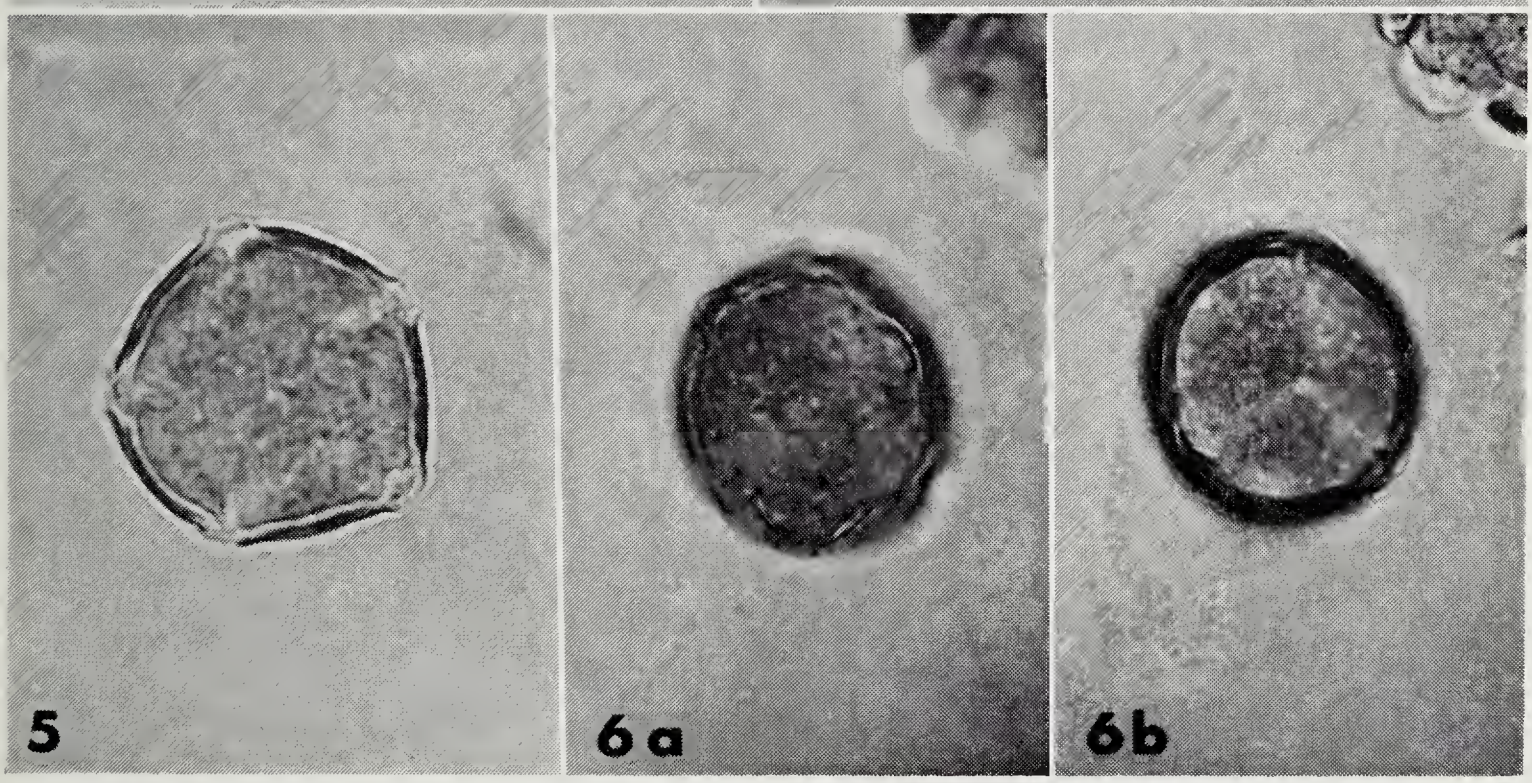
The comparison of percentages of tritreme and tetratreme pollen grains in the diploids and tetraploids provides a clear distinction. The predominance of the tritreme class in all of the diploids (72.6-89.1%) shifts to a predominance of the tetratreme class (71.8-72.3%) in the two tetraploids examined. The doubling of the somatic chromosome number appears to result in the addition of one pore in the majority of pollen grains and the change occurs with sufficient regularity to provide a reliable means of establishing somatic chromosome numbers from the examination of pollen grains if only diploids and tetraploids are involved.

The hexaploid 'Eldorado' pore number classification shows the predominant pore class to be tetratreme. As with the tetraploids, the tetratreme percentage readily separates the hexaploid from all of the diploids, but no significant differences are apparent between the hexaploid 'Eldorado' and the tetraploid red raspberries, 'Malling Exploit' and 'Colossus'. All three have varying percentages of ditreme, tritreme, and pentatreme pollen in addition to the predominant tetratreme type. The percentage of the hexatreme pollen for the tetraploid 'Malling Exploit' was higher than that found for the hexaploid 'Eldorado' and no hexatreme pollen was found in the samples of tetraploid 'Colossus'. Obviously, the pore number classifies the hexaploid as a polyploid, but no further separation is possible from the evidence obtained in this study.

The evidence does suggest, however, that a more definitive study of *Rubus* pollen morphology, including varying levels of ploidy in a complete range from diploid through octoploid and higher, could provide information that would be helpful in estimating somatic chromosome number rapidly.







FIGURES 1-6b.—Pore numbers in pollen grains of *Rubus*: 1. Monotreme. X 1250; 2. Ditreme. X 1250; 3. Tritreme, the predominant type of diploid forms. X 1600; 4. Tetratreme, the predominant type in tetraploid and hexaploid forms. X 1600; 5. Pentatreme. X 1250; 6a, 6b. Optical sections of a pollen grain of the hexatreme class. X 1250.

TABLE 1.—Pore Number Classification of Diploid, Tetraploid, and Hexaploid Pollen from *Rubus* Cultivars and Selections.

Cultivar or Selection[1]	Ploidy	No. Grains Scored	% Grains/Class (-treme)					
			Mono-	Di-	Tri-	Tetra-	Penta-	Hexa-
Red Raspberries								
'Taylor'	2n	70	1.4	10.0	88.6	.....	.....	.....
NC-223	2n	67	1.5	3.0	82.1	7.5	6.0	.....
'Malling Exploit'	4n	65	.....	1.5	9.2	72.3	4.6	12.3
'Colossus'	4n	71	.....	7.0	15.5	71.8	5.6	.....
Black Raspberry								
'Blackhawk'	2n	73	.....	2.7	72.6	21.9	1.4	1.4
Purple Raspberry								
NC-50-13-1	2n	73	4.1	11.0	75.3	8.2	1.4	.....
Erect Blackberry								
'Eldorado'	2n	64	4.7	.....	89.1	6.3	.....	.....
'Eldorado'	6n	72	.....	1.4	18.1	70.8	2.8	6.9

[1] The genus *Rubus* is notorious for its difficult taxonomy and unstable nomenclature. These cultivars and selections are best described as species hybrids derived from at least the following: *R. idaeus, R. strigosus, R. parvifolius, R. occidentalis,* and *R. allegheniensis.*

ACKNOWLEDGMENTS

The authors express thanks to Dr. J. W. Hull, Small Fruit Research Center, Southern Illinois University, Carbondale, Illinois, for providing a source for some of the pollen.

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Manuscript received January 8, 1968.

NOTES

FREE SWIMMING ZOOSPORES OF SORASTRUM

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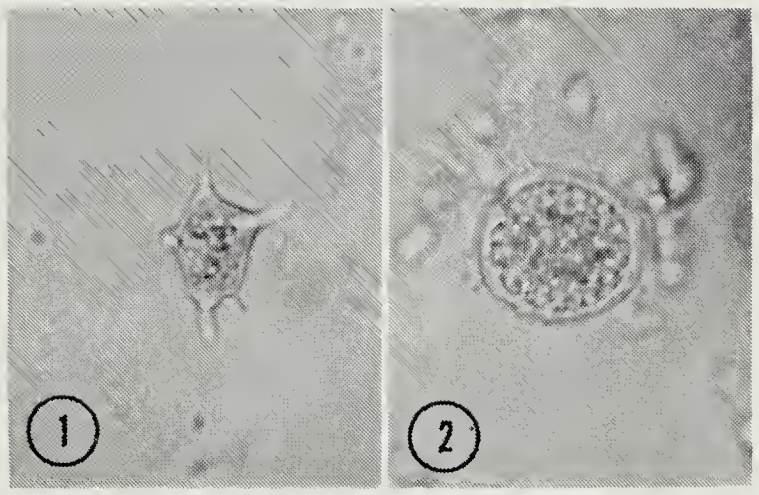
ABSTRACT. — Free swimming zoospores in a *Sorastrum* culture developed into spherical cells with five spines. When transferred to a fresh mineral medium, the spined cells reproduced typical *Sorastrum* colonies.

The colonial green fresh water alga *Sorastrum* had been maintained for three years in a soil water culture. The medium for this culture was prepared in a 250 ml Erlenmeyer flask by covering a small amount of $CaCO_3$ with one inch of sterile garden soil and then adding enough sterile distilled water to cover the soil by one inch. The flask was inoculated with a *Sorastrum* culture (LB785) from the Culture Collection of Algae at Indiana University, and then placed under fluorescent lights of 350 ftC intensity with a 16-8 hr light-dark cycle. Temperature had been maintained at 22-24 C for three years, but was held at 20 C during the fourth year. Sterile distilled water was added as needed to offset evaporation.

During the fourth year the liquid of the culture was carefully poured off, and the soil was allowed to dry somewhat. Three months later sterile distilled water was added so that the original liquid level was restored. Daily examination of the culture for two weeks after the water addition, and thence once weekly for 6 months, revealed many five-spined cells with either thick or thin walls, some free swimming cells, and some typical *Sorastrum* colonies. Since the free swimming and the 5-spined cells have not been reported before for this genus, the author decided to follow their development.

Free swimming cells (zoospores) were completely green except for a clear area from which two anterior flagella of equal length emerged. The zoospores had no cell wall, but were bounded by a thin membrane. The zoospores moved rapidly through the liquid, and at a rate faster than the author could follow with a 45X objective and a mechanical stage. Fast moving zoospores elongated somewhat, but they became spherical when their movement slowed. The zoospores eventually stopped; the length of time they were motile was not determined. After stopping each cell became angular, and five sharp projections appeared on its membrane. During the next 24 hours the cell rounded and secreted a wall; and the projections elongated into 5 spines (FIG. 1). The spines were identical and evenly spaced on the cell. After two weeks each 5-spined spherical cell had enlarged considerably (FIG. 2). When such cells were allowed to remain in the liquid of the soil water flask, they developed thick walls of many layers. Such cells often became yellow to orange, and frequently contained several orange fat droplets. Upon transfer to fresh mineral medium C (Davis 1967) each 5-spined cell divided internally, and released a spherical vesicle containing 8-16 zoospores. After they stopped swimming, each developed into a cell with 4 outwardly-pointing spines and a stipe pointing toward the center of the vesicle.



The stipes of all the cells united, and a typical *Sorastrum* colony resulted.

Discussion

Each cell of a *Sorastrum* colony can reproduce a daughter colony by releasing a vesicle with biflagellate zoospores; each of these becomes a colony cell which produces four outwardly pointing spines and a blunt stipe which points toward the center of the vesicle and fuses with the stipes of the other cells of the same colony (Davis 1966). From their appearance and reproduction, the author believes that the 5-spined cells herein reported originated from zoospores which had escaped from their vesicle. Development of the escaped zoospores in the culture medium and away from the usual confining conditions in the vesicle may account for the five identical spines and the wide spacing of the spines on the single cells.

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Manuscript received January 29, 1968.

RANGE EXTENSION OF THE EASTERN SPADEFOOT TOAD (*SCAPHIOPUS HOLBROOKI*, HARLAN) IN SOUTHERN ILLINOIS

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ABSTRACT. — An extension of the usual range of the spadefoot toad is reported.

The Eastern Spadefoot Toad (*Scaphiopus holbrooki*) is known in Illinois only from a few specimens taken along the Mississippi River in Alexander, Union and Monroe counties; and one locality in southwestern Johnson County. The presumed range in Illinois according to Smith (1961) includes extreme southern Illinois south of a line from roughly Murphysboro to Golconda.

A single specimen was collected on August 11, 1967, near Lusk Creek in northcentral Pope County. This was slightly north of the presumed range and some 30 miles ENE of the known range representing an extension into the Shawnee Hill uplands.

The specimen was captured in an old limestone quarry near the center of the SE¼ of Sec. 16, T12S, R6E. The toad was captured overnight in a Museum Special snap trap set at the base of the 20-foot high quarry wall. The toad, caught across the thighs, was still alive when removed from the trap. Apparently the toad had attempted to capture ants feeding on the oat and peanut butter bait on the trap and sprung the trigger.

The soil along the base of the quarry wall was reddish with a loose sandy texture and void of any vegetation except for a few shrubby hardwood trees. The surrounding hillside was an upland oak-hickory association.

LITERATURE CITED

SMITH, P. W. 1961. The amphibians and reptiles of Illinois. Ill. Nat. Hist. Sur. Bull. 28(1).

Manuscript received October 27, 1967.

THE EFFECT OF TEMPERATURE AND VITAMIN B_{12} ON THE GROWTH OF *COELASTRUM MICROPORUM* NAEG. ISOLATES

G. KAISER, D. L. LYNCH, M. J. STARZYK and M. G. FENWICK
Northern Illinois University, DeKalb, Illinois

ABSTRACT. — A study was made on the affect of temperature and Vitamin B_{12} concentration on the growth of strains 280 and 281 of the green alga, *Coelastrum microporum* Naeg. Optimum temperatures for growth differed slightly for the 2 strains. However, when combined with Vitamin B_{12} treatment, major growth differences became apparent. The most striking of these differences occurred with strain 280.

Previous comparative work on *Coelastrum microporum* Naeg. Gates *et al.* (1966) and Lynch *et al.* (1967) has indicated differences between strains 280 and 281 (University of Indiana Algal Coll.). Using coenobial formation as a criterion it was established that these strains differed in their response to gibberellic acid while colchicine and increased solidity of the medium had an inhibitory effect on colonial development.

The purpose of the current study was to elaborate on other differences by establishing the effects of temperature and Vitamin B_{12} (cobolamine) concentration on the growth of strains 280 and 281 of *Coelastrum microporum*.

METHODS AND MATERIALS

Both isolates of *Coelastrum* were grown in Bristol's medium as modified by Bold with glucose added at the rate of 10g/liter. After each isolate was sufficiently grown out, the cells were spun down, washed with sterile distilled water, recentrifuged and resuspended in sterile distilled water. This constituted the inoculum used throughout the experiment. In the experimental design each isolate was inoculated into 4 tubes of modified Bristol's medium, 4 tubes of modified Bristol's medium plus 3 μg/ml Vitamin B_{12} concentrate and 4 tubes of modified Bristol's medium plus 10 μg/ml Vitamin B_{12} concentrate. All tubes were placed in a Sherer growth chamber having 16 hours of illumination and a night temperature fluctuation of 10°C. less than the experimental temperature. Each set of tubes for isolates, 280 and 281 of *C. microporum*, was incubated for 5 days. Experimental temperatures used were 20°C., 28°C., 34°C., and 43°C., daylight readings. The amount of growth was estimated by transmission readings on a Bausch and Lomb "Spectronic-20" colorimeter at 520 mμ.

RESULTS AND DISCUSSION

In terms of temperature requirements (TABLE 1) optimum growth of isolate 280 occurred at both 28° and 34°C. Isolate 281 grew best at 28°C. but did grow somewhat better at 34° than 20°C. Neither isolate 280 nor 281 grew at 43°C. under any conditions.

Incorporation of 3 μg/ml Vitamin B_{12} concentrate into the medium improved the growth response of isolate 280 at both 28° and 34°C., the best growth occurring at 34°C. Additional Vitamin B_{12} concentrate (10 μg/ml) significantly improved growth of isolate 280 at 20°C. in relation to the control and the 3 μg/ml treatment, but did not influence previously altered growth rates of the lower (3 μg/ml) Vitamin B_{12} concentrate treatment at the other temperatures. Isolate 281 was affected similarly by the incorporation of Vitamin B_{12} concentrate into the test medium. Significant increases in growth occurred at 28° and 34°C.

TABLE 1. Growth Response of Strains 280 and 281 of *Coelastrum microporum* to Temperature and Vitamin B_{12} Treatment.

	20°C.	28°C.	34°C.	43°C.
	% Transmittance*			
STRAIN 280				
Control	87.2	56.5	55.5	N.G.
3 μg/ml B_{12} Conc.**	88.2	50.0	46.2	N.G.
10 μg/ml B_{12} Conc.	64.5	49.7	45.5	N.G.
STRAIN 281				
Control	89.6	55.2	64.5	N.G.
3 μg/ml B_{12} Conc.	89.0	50.7	55.5	N.G.
10 μg/ml B_{12} Conc.	88.7	50.7	56.0	N.G.

* Average percent transmittance of 4 replicates.
** The Cobolamine concentrate contained 3,000 micrograms of B_{12}/g

upon the addition of 3 μg/ml Vitamin B_{12} concentrate with the best growth occurring at 28°C. No additional increase in growth at the 10 μg/ml level of the vitamin was observed. No stimulatory effect of 10 μg/ml of Vitamin B_{12} concentrate took place with 281 at 20°C. as was previously noted for 280.

Differing growth response interactions (temperature and Vitamin B_{12} combinations) were observed with 2 isolates of the green alga, *C. microporum* Naeg.

LITERATURE CITED

GATES, J., J. ANDERSON, D. L. LYNCH and M. G. FENWICK. 1966. Some Physiological Studies on Two Strains of the Green Alga *Coelastrum microporum* Naeg. Trans. Ill. State Acad. of Sci. 59:399-400.

LYNCH, D. L., M. G. FENWICK and L. O. HANSEN. 1967. Heterotrophic Nutrition in the Genus Coelastrum Naeg. Trans. Amer. Microsc. Soc. 86:499-502.

Manuscript received April 15, 1968.

SOME COMPOSITAE NEW TO ILLINOIS

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Department of Botany, Southern Illinois University

ABSTRACT. — *Cosmos sulphureus* Cav., *Matricaria maritima* L. var. *agrestis* (knaf) Wilmott, *Coreopsis basalis* (Otto & Dietr.) Blake, *Leontodon leysseri* (Wallr.) G. Beck, *Eupatorium perfoliatum* L. forma *trifolium* Fassett, and *Cirsium discolor* (Muhl.) Spreng. forma *albiflorum* (Britt.) House are here for the first time reported as occurring in Illinois.

This paper reports six taxa of Compositæ previously unknown as occurring in Illinois. It is the result of recent herbaria studies by the author in preparation of the Compositæ in the *Illustrated Flora of Illinois*, edited by Robert H. Mohlenbrock, Southern Illinois University.

Cosmos sulphureus Cav. — A native of Mexico, Central America, and northern South America, this species is cultivated and has become established in the West Indies and southern United States north to the New England region. This plant is known from Illinois on the basis of a single collection deposited in the herbarium of the Illinois State Museum: WILL CO.: 2 mi. W. of Blodgett, *Fuller 11226*. Whether this collection represents an escape from cultivation or an introduction by man by means of some mode of transportation (railway or truck) enroute from the south remains only a matter of speculation. The persistance of this southern species in this northern climate is questionable and the inclusion of it in a flora of Illinois also is a matter of debate.

Matricaria maritima L. var. *agrestis* (Knaf) Wilmott. — This taxon, a native of Europe, is established in eastern United States and Canada as well as on the Pacific coast. Variety *maritima* is found occasionally in saline areas along the eastern coast and differs from var. *agrestis* in being more branching, usually perennial, and having broader leaf segments. There is evidence that it is merely an ecological form and thus the taxonomic status of the varieties of this species are open to question, but it is not the intent of the author to discuss its status in this paper. This plant is known from Illinois from the following collection deposited in the herbarium of Southern Illinois University: DU PAGE CO.: C. Armbrust residence, Norfolk St., Downers Grove, *Armbrust s.n.*

Coreopsis basalis (Otto & Dietr.) Blake. — This species is a native of Texas, although it sometimes occurs as an escape from cultivation elsewhere. It is known from Illinois from a single collection deposited in the herbarium of the Illinois State Museum: LAKE CO.: Cedar Lake, Lake Villa, *Fuller 13272*.

Leontodon leysseri (Wallr.) G. Beck— This species, a native of Europe, is sparingly adventive in the New England area west of Ohio. It is the second species of this genus now reported from Illinois. *Leontodon autumnalis* L., also adventive from Europe, was reported once by Pepoon (Bull. Chicago Acad. Sci. 8:1-554, 1927) but has not been found since. The following specimen sent to Robert H. Mohlenbrock by Mr. Floyd Swink, Morton Arboretum, has been verified by the author and is deposited in the herbarium at Southern Illinois University: COOK CO.: Mount Hope Cemetery just north of Blue Island, *Bartel s.n.*

Eupatorium perfoliatum L. forma *trifolium* Fassett. — This form differs from the typical *Eupatorium perfoliatum* in having three leaves at a node rather than two. It previously was known only from Quebec in Canada and Maine and Massachusetts in the United States. The

following collection is deposited in the herbarium at the Field Museum of Natural History, Chicago: COOK CO.: Palos Park, *Steyermark 40956*.

Cirsium discolor (Muhl.) Spreng. forma *albiflorum* (Britt.) House.—This form differs from the typical *Cirsium discolor* in having the flowers white in color rather than purple. It occurs sporradically throughout the range of the species. The following collection is deposited in the herbarium at Southern Illinois University: LA SALLE CO.: 2 mi. NW of Mendota along IC railroad tracks, *Richardson 646*.

Manuscript received December 27, 1967.

ACADEMY BUSINESS

SECRETARY'S REPORT FOR THE YEAR — APRIL 29, 1967 - APRIL 26, 1968

COUNCIL MEETINGS

The Council held four meetings during the year, as stipulated in the Constitution.

First Council Meeting. — The first Council meeting was held at Eastern Illinois University, on Saturday morning, April 29, with President Milton D. Thompson presiding. Eleven persons were present.

The following persons were appointed to serve as chairmen for the committees listed below:

Elnore Stoldt, Junior Academy; Joan Hunter, Planning; Fred Rolf, for the newly created Visiting Lecturer Committee. The Council voted to disband the Educational Films Evaluation Committee, and approved the selection of William J. Probst as Second Vice-President for 1967-1968. Also, the Treasurer was made an ex officio member of the Visiting Lecturer, Budget, Legislation and Finance, and Permanent Central Office Committees.

Second Council Meeting. — This was held at the Illinois State Museum, Springfield, on Saturday morning, November 4, 1967. Eighteen members were present.

President Thompson read a letter from W. Klimstra, First Vice-President, asking that he be replaced. The Council unanimously voted to appoint William C. Ashby as the new First Vice-President.

The Council appointed Emily Blasingham as Chairman of the Archæological and Historical Sites Committee and also drafted a resolution regarding the proposed Oakley reservoir (see Resolution Number Six of the Annual Business Meeting).

Third Council Meeting. — This was held at the Illinois State Museum, Springfield, on Saturday morning, February 17, 1968. Twenty-three members were present.

The Council appointed Walter B. Welch as Chairman of the Nominations Committee. William Probst, Second Vice-President, suggested that future section chairmen be given a small budget to help defray the expenses that are incurred in preparing a program. The possibility of revising the current format of events during the annual meetings was also discussed, but action on this matter was postponed for later discussion by the new officers and council members who would be elected at the Annual Business Meeting in April.

Fourth Council Meeting. — This meeting was held at Rusty's Restaurant, Edwardsville, on Thursday evening, April 25, 1968, following the Council Dinner. Twenty-two members were present.

The Council passed a motion to insert into the Academy budget an allowance of $1000 for the Permanent Central Office Committee so that it could begin its task of contacting various philanthropic organizations. President Thompson, reporting for the Librarian, stated that current cost of publishing the *Transactions* was more than the amount of money budgeted at the beginning of the biennium.

The Council passed a motion to authorize the Legislation and Finance Committee, or its successor, to use up to $5000 of the Academy's reserve funds to begin an investment program.

THE 61ST ANNUAL MEETING

General Meeting. — The general session of Friday morning, April 26, 1968, held in the Science Auditorium, was called to order by President Thompson.

Dr. Lawrence R. McAneny, Dean of Science and Technology Division at Southern Illinois University, Edwardsville, gave the address of welcome. The presidential address, "The Impact of Science in a Democracy," was followed by the panel discussion on "Maintaining Quality Water in Illinois." Dr. Robert H. Harmeson, Chemistry Section, Illinois State Water Survey, Urbana, discussed "Available Water Resources of Illinois," and Mr. R. E. Favreau, Illinois Department of Public Health, Carbondale, discussed the "Action Program to Secure Quality Water for Illinois."

On Friday evening, in the University Center, Dr. Mary Alice McWhinnie, Department of Biological Sciences, DePaul University, Chicago, gave the Annual Public Lecture, "Science, Technology, and Antarctica."

On Saturday morning, April 27, Drs. Paul D. Kilburn and John Wanamaker of Principia College conducted a botany field trip to the bluffs along the Great River Road north of Alton. Drs. Emily Blasingham of the Illinois State Museum, Robert Hall of Marquette University, and Charles J. Bareis of the University of Illinois led an archæological field trip to Cahokia.

Technical Sessions. — On Friday afternoon, April 26, the following papers were presented:

ANTHROPOLOGY

Room 0186, Communications Building
Kent Schneider, Chairman
Knox College, Galesburg

Field Reconnaissance in Mauritania. Patrick J. Munson, University of Illinois, Urbana.

Aboriginal Use and Occupancy of the American Bottom. Alan D. Harn, Dickson Mounds Museum, Lewistown.

A Preliminary Report of the Excavation of the Millbrook Site, Peoria County, Illinois. Kent Schneider, Knox College, Galesburg.

Hotel Plaza (Lsᵛ 36, Ceramics: A Typological Study. Gail D. Schroeder, Illinois State Museum, Springfield.

The Maya Potter as a Scientist. Dean E. Arnold, University of Illinois, Urbana.

Socio-political Problems of Archæology in the Middle East: Hebron, a Case in Point. Martin McGuire, Illinois State University, Normal.

The Importance of Cahokia in Midwest Prehistory. Robert L. Hall, Marquette University, Milwaukee, Wisconsin.

The Subsistence Economy of the Illinois Indians. Emily J. Blasingham, Illinois State Museum, Springfield.

AQUATIC BIOLOGY

Room 0410, Peck Classroom Building
L. Stephen Whitley, Chairman
Eastern Illinois University, Charleston

A Survey of Piasa Creek. Stephen Smith, Southern Illinois University, Edwardsville.

The Recovery of an Illinois Stream from Cyanide Poisoning. Leonard Durham, Eastern Illinois University, Charleston.

Primary Productivity in an Illinois River Flood Plain Lake. B. J. Mathis and S. Z. Taylor, Bradley University, Peoria.

The Role of Energy Production in the Toxicity of Several Pollutants to Bluegill. Robert C. Hildebran, Illinois Natural History Survey, Urbana.

Age and Rate of Growth of Bluegills in Reelfoot Lake. Robert J. Schoffman, Griffin High School, Springfield.

Biology of Two Sibling Species of Fundulus in Southern Illinois. Jamie E. Thomerson, Southern Illinois University, Edwardsville.

The Biology of the Tubificidæ of Illinois. Stephen Whitley, Eastern Illinois University, Charleston.

BOTANY
Section A

Room 2224, Science Building
Aristotel J. Pappelis, Chairman
Southern Illinois University, Carbondale

Changes in the Nuclear Dry Mass of Onion (*Allium cepa*) Epidermal Cells Following Exposure. Paraskevi BeMiller, W. S. Courtis, and A. J. Pappelis, Southern Illinois University, Carbondale.

Degenerative Nuclear Changes of Onion (*Allium cepa*) Epidermal Cells as Studied with the Interference Microscope. Paraskevi BeMiller, W. S. Courtis, and A. J. Pappelis, Southern Illinois University, Carbondale.

The Genetics and Histology of Postzygotic Lethality in *Zea mays*. Delano K. Cox, Illinois State University, Normal.

Tree Ring Chronologies of Three Woody Species in the Central Mississippi Valley. Eugene T. Estes, Rend Lake College, Mt. Vernon.

The Influence of Gold and Chemical Treatment on the Afterripening of Apple Seeds. Bernard E. Colvis and James B. Mowry, Southern Illinois University, Carbondale.

A 27:37 Ratio in *Zea* and Resultant Problems. J. Louis Martens, Illinois State University, Normal.

A Study of the Effect of Plant Transpiration Retardants on Field Corn. Neil A. Miller, Southern Illinois University, Carbondale.

Evapotranspiration Contrast Between South-facing Hill Prairies and North-facing Forest. Christopher R. Ranft and Paul D. Kilburn, Principia College, Elsah.

On the Effects of DMSO in Cation Transport by Excised Barley Roots. Walter E. Schmid, Southern Illinois University, Carbondale.

Further Studies on Thallium and its Absorption by Excised Barley Roots. Walter E. Schmid and G. Craig Colclasure, Southern Illinois University, Carbondale.

Studies on the Physiology of Sugar Cane in Southern Illinois. Charles E. Bare, Walter E. Schmid, and A. J. Pappelis, Southern Illinois University, Carbondale.

Section B

Room 3225, Science Building
Richard C. Keating, Chairman
Southern Illinois University, Edwardsville

Soil Microfungi of a Prairie Restoration Area. Philip G. Eickhoff and James H. Grosklags, Wheaton High School, Wheaton, and Northern Illinois University, DeKalb.

Cytological Observations of *Clarkia* Interspecific Hybrids. Fathi Abdel-Hameed, Northern Illinois University, DeKalb.

Succession and Species Distribution in Relation to Erosion in Abandoned Fields. Fakhri A. Bazzaz, University of Illinois, Urbana.

The Asclepiadaceæ of Illinois — A Photographic Essay. Carl W. Bollwinkel and Harold R. Hungerford, Southern Illinois University, Carbondale.

Comparative Anatomy of the Flower in the Cochlospermaceæ and Bixaceæ. Richard C. Keating, Southern Illinois University, Edwardsville.

Trends of Specialization in the Stipe Anatomy of *Dennstædtia* and Related Genera. Richard C. Keating, Southern Illinois University, Edwardsville.

Plant Distribution on Hills Surrounding Kabul, Afghanistan. M. L. Kumler, Southern Illinois University, Edwardsville.

Additions to the Chesterian Flora of Southern Illinois. James Jennings and Lawrence C. Matten, Southern Illinois University, Carbondale.

Identity of *Arnoldella* and *Kalymma*. Wade S. Sebby and Lawrence C. Matten, Cornell University, Ithaca, New York, and Southern Illinois University, Carbondale.

Recent Additions to the Algal Flora of Illinois: The Genus *Phacus* Dujardin. Kenneth L. Weik, Lake Forest College, Lake Forest.

CHEMISTRY

Emanuel Merdinger, Chairman
Roosevelt University, Chicago

Section A

Room 2405, Peck Classroom Building
Leon L. Gershbein, Presiding
Northwest Institute for Medical Research, Chicago

Lipids of *Pullularia pullulans*. Roy C. McClain and Emanuel Merdinger, Roosevelt University, Chicago.

Retention and Distribution of C^{14} from Glucose-1-C^{14} in the Cell Extracts of *Pullularia pullulans*. Emanuel Merdinger, Roosevelt University, Chicago.

Isolation and Chemistry of M-protein of Group A Type 12 Streptococci. Charles F. Lange, Hektoen Institute, and Rosita Lee, Roosevelt University, Chicago.

Some Molecular Aspects of Biosynthesis and Synthesis of Immunoglobulins by Polyribosomes. Milton J. Becker, Biomedical Institute for Research, Chicago.

Rat Liver Phosphorus Metabolism and Parathyroid Hormone Extract. Vincent Sawinski, Chicago City College, and Louis J. Blanchet, Loyola University, Chicago.

Disc Electrophoresis of Tissue and Fluid Pre-Albumin Components. Leon L. Gershbein, Northwest Institute for Medical Research, Chicago.

Inhibition of Yeast Ureidoglycolatase. Virginia V. Smith, Nina Spalek, and Allan H. Roush, Illinois Institute of Technology, Chicago.

A Study of the Chloride-bicarbonate Shift, Illustrating the Donnan Membrane Effect in Vertebrate Blood Cells. Robert Dons, Gale Van Wingerden, and Frank O. Green, Wheaton College, Wheaton.

Plasma and Cerebrospinal Fluid Amino Acid in Hepatic Coma. Donald A. Silverman, A. Dubin and F. Steigmann, Hektoen Institute for Medical Research, Chicago.

Radiorespirometry — A Study of the Glucose Metabolism of the Japanese

Quail. Frank O. Green, Wheaton College, Wheaton.

Homology of Old World and New World Primate Cytochrome C. Saul B. Needleman, Northwestern University Medical School and V. A. Research Hospital, Chicago.

Section B

Room 2409, Peck Classroom Building
Carl Weatherbee, Presiding
Millikin University, Decatur

The Synthesis and Study of Aromatic Gem-Dichlorocompounds. Robert C. Duty, Illinois State University, Normal.

Solubility Products for Some Transition Metal Oxinates. R. Barry Dodson and R. E. Van Atta, Southern Illinois University, Carbondale.

Mannich Condensations of Thiophenols and Primary Aliphatic Amines. Carl Weatherbee, Jerry W. Nieft, and Gregory C. Munie, Millikin University, Decatur.

Phosphates in Peoria Lake: A Quantitative and Qualitative Evaluation of a Nutrient in Natural Waters. William T. Sullivan and David W. Hullinger, Illinois State Water Survey, Peoria.

A Review of Absolute Asymmetric Syntheses. Giles Lee Henderson, Eastern Illinois University, Charleston.

Water Pollution — A Study of Water from a Sanitary Landfill in Northeastern Illinois. Paul McGinnes and Frank O. Green, Wheaton College, Wheaton.

Oxygen Absorption by Coal. Some Observations on Oil Treatment. G. R. Yohe, Illinois State Geological Survey, Urbana.

Monoesters of Phenolphthalein. C. R. Dorn and W. M. Hoehn, G. D. Searle & Co., Skokie.

Association Constants by Non-Linear Least Squares. Boris Musulin, Southern Illinois University, Carbondale.

Interaction Potential Constants of Some Oxygen Containing Bent-Triatomic Molecules. Ralph L. Bain, Southern Illinois University, Edwardsville.

Preparation of 6-alkyl-6-cyano-2-phenylcyclohexanones. D. P. Logan, W. J. Probst, and S. E. Wilson, Southern Illinois University, Edwardsville.

The Reaction of Styrene with N, N-Dibromobenzenesulfonamide. G. K. Baker, D. P. Davis, and W. J. Probst, Southern Illinois University, Edwardsville.

The Reaction of 1, 1-Diarylethylenes with N-Bromoacetamide. W. J. Probst and T. A. Schmidt, Southern Illinois University, Edwardsville.

A Modern Look at the Glockler Formula. Victor Fong and Boris Musulin, Southern Illinois University, Carbondale.

Section C

Room 2412, Peck Classroom Building
Mary Marina Kennelly B.V.M., Presiding
Mundelein College, Chicago

Clinical Chemistry's Contribution to Surgery. G. E. Batayias, R. R. Martins, and Robert A. Scheidt, St. Luke's Hospital, Milwaukee, Wisconsin.

Homology of *Pseudomonas* Cytochrome c-551 with other Mammalian c-type Cytochromes. Saul B. Needleman, Northwestern University Medical School and V. A. Research Hospital, Chicago.

The Effect of Hexadimethrine Bromide (Polybrene) on Kidney Function in Dogs. Richard A. Collins and Robert A. Scheidt, St. Luke's Hospital, Milwaukee, Wisconsin.

Membrane Alteration in p-Fluorophenylalanine treated *Escherichia coli*. Margaret Brostrom, and Stephen B. Binkley, University of Illinois at the Medical Center, Chicago.

The Effect of Hexadimethrine Bromide (Polybrene) on the Blood Coagulative Mechanism. Richard A. Collins and Robert A. Scheidt, St. Luke's Hospital, Milwaukee, Wisconsin.

Fertility Control with Hormonal Steroids. Richard E. Ray and Byron Riegel, G. D. Searle & Co., Skokie.

Magnetic Moments of Metal Complexes by NMR. Sister Mary Marina Kennelly B.V.M., Mundelein College, Chicago.

Dehydration of 2-Methylcyclohexanol and Related Compounds. A. Donald Glover, Bradley University, Peoria.

Reaction of Benzoyl Peroxide with Furan. W. Black and K. E. Kolb, Bradley University, Peoria.

Iodine-Alkene Equilibrium. D. Hunsicker and K. E. Kolb, Bradley University, Peoria.

CONSERVATION

Room 2413, Peck Classroom Building
Richard D. Andrews, Chairman
Eastern Illinois University, Charleston

Nesting Ecology of the Prairie Chickens in Illinois. Ronald L. Westemeier, Illinois Natural History Survey, Urbana.

The Effect of Large Dams on Wildlife. Hurst H. Shoemaker, University of Illinois, Urbana.

Forest Types of Lusk Creek in Pope County, Illinois. William C. Ashby, Southern Illinois University, Carbondale.

Lusk Creek, Pope County, Illinois, as a Natural and Scientific Area. John Schwegman, Southern Illinois University, Carbondale.

Habitat Preservation in Britain. Paul Kilburn, Principia College, Elsah.

Bobwhite Whistling and Population Levels. Keith P. Thomas, Illinois Natural History Survey, Urbana.

Vegetation of the Illinois Beach State Park. Elizabeth T. Lunn, Lake Forest College, Lake Forest.

Age-related Mortality of Illinois Pheasants in Fall and Winter. Stanley L. Etter, Illinois Natural History Survey, Urbana.

GEOGRAPHY

Robert L. Koepke, Chairman
Southern Illinois University, Edwardsville

Section A

Manufacturing Geography
Room 3305, Peck Classroom Building
Robert L. Koepke, Presiding

Measurement in Manufacturing Geography: Criterion of Manufacturing. Raymond and Virginia Waxmonsky, University of Illinois, Urbana.

Changes in Manufacturing in Illinois. James E. Patterson, Illinois State University, Normal.

Manufacturing in the Chicago Standard Metropolitan Statistical Area. Martin W. Reinemann, Northern Illinois University, DeKalb.

A Porton of the St. Louis North Broadway Industrial District: A Study in Obsolescence. Thomas G. Tucker, Southern Illinois University, Edwardsville.

Linkage in Chemical Manufacturing in Southern West Virginia. David W. Ganyard, Western Illinois University, Macomb.

Section B

Transportation Geography
Room 3313, Peck Classroom Building
Edwin H. Draine, Presiding
University of Illinois, Chicago Circle

Some Comments on Soviet Commercial Air Transportation. B. Ross Guest, Northern Illinois University, DeKalb.

Technical Innovation and Planned Highway Development in Tropical Australia. Allan R. McNeill, Illinois State University, Normal.

A Systems Approach to Transportation Geography. Ross D. MacKinnon, Northwestern University, Evanston.

Theoretical Constructs and Empirical Comparisons of Some Measures of Accessibility in Transportation Networks. Frederick P. Stutz, Northwestern University, Evanston.

Spatial Repetition and Urban Travel Behavior. Sophia Bowlby, Northwestern University, Evanston.

Section C

Physical Geography
Room 3315, Peck Classroom Building
Kenneth R. Martin, Presiding
Western Illinois University, Macomb

Certain Aspects of Karst Depression Distribution in Northern Puerto Rico. Placido D. LaValle, University of Illinois, Urbana.

Geographical Approaches to 7th Approximation Subgroup Analysis of Selected Soil Interpretation Maps. Paul W. Mausel, Eastern Illinois University, Charleston.

Computed Soil Moisture Patterns In and Around the Prairie Peninsula During the Great Drought of 1933-1934. A. Steven Messenger, Northern Illinois University, DeKalb.

The Effect of Fire on Ecological Productivity in the Flint Hills of Kansas. Ronald Isaac, Southern Illinois University, Carbondale.

Physical and Social Factors Affecting Woodland Fire Incidence. Vernon Meentemeyer, Southern Illinois University, Carbondale.

Section D

Economic Geography
Room 3305, Peck Classroom Building
Harry B. Kircher, Presiding
Southern Illinois University, Edwardsville

A Multi-variable Approach to the Delineation of Economic Regions in Illinois. Robert B. Douglas, Bradley University, Peoria.

The Office of Minerals Exploration: A Change in the Geography of Minerals? Bruce W. Smith, University of Illinois, Urbana.

Shrimp Cities, U.S.A.: Brownsville and Port Isabel, Texas. C. Daniel Dillman, Northern Illinois University, DeKalb.

Section E

Cultural Geography
Room 3313, Peck Classroom Building
Dalias A. Price, Presiding
Eastern Illinois University, Charleston

Bishop Hill: A Swedish Pioneer Colony in Illinois. Ronald E. Nelson, Western Illinois University, Macomb.

Toward a Noneconomic Emphasis as Cultural Geography. John A. Jakle, University of Illinois, Urbana.

Some Early Maps of the Middle Mississippi Valley. William B. Baker, Southern Illinois University, Edwardsville.

GEOLOGY

Room 0312, Peck Classroom Building
Dorothy J. Gore, Chairman
Southern Illinois University, Edwardsville

Identification of Mafic Dikes of Different Ages, Mount Desert Island, Maine. Ramakant P. Agaste, University of Illinois and Illinois State Geological Survey, Urbana.

Eridophyllum in the Middle Devonian of Southern Illinois and Southeastern Missouri. George H. Fraunfelter, Southern Illinois University, Carbondale.

Techniques in the Preparation of a Fragmented Mastodon Skull. William M. Johnson, Augustana College, Rock Island.

Engineering Geology of the Six Mile Creek Damsite. Paul B. DuMontelle, Herbert D. Glass, and John P. Kempton, Illinois State Geological Survey, Urbana.

Sedimentation and Consolidation of Fine-grained Sediments. Arthur White, Illinois State Geological Survey, Urbana.

METEOROLOGY AND CLIMATOLOGY

Room 3422, Peck Classroom Building
W. L. Denmark and W. J. Roberts, Co-Chairmen
Urbana, Illinois

Satellite and Mesometeorology Research. Dorothy L. Bradbury, University of Chicago, Chicago.

A Speculative Weather Modification Scheme for Illinois. Richard G. Semonin, Illinois State Water Survey, Urbana.

Temperature Distributions of the Initial Radar Echoes of Clouds that Occurred within a 60-mile radius of West Plains, Missouri, During the Summers 1960-62. Miss Maureen Dungey, University of Chicago, Chicago.

A Method for Predicting Rainfall Rate-Radar Reflectivity Relationships. Robert Cataneo, Illinois State Water Survey, Urbana.

Collection of Cloud Particles by Using Replication Technique in Natural Clouds. Paul Spyers-Duran, University of Chicago, Chicago.

A Statistical Evaluation of Hail Modification Experiments. Paul T. Schickedanz and Stanley A. Chagnon, Jr., Illinois State Water Survey, Urbana.

MICROBIOLOGY

Room 1225, Science Building
Lloyd D. Witter, Chairman
University of Illinois, Urbana

A Quick Method for Preparing Plant Viruses and Particles for Detection in the Electron Microscope. Mary Ruth Thompson, Department of Plant Pathology, University of Illinois, Urbana.

Ultrastructure of Wheat Tissue Infected with Soil-borne Wheat Mosaic Virus. Margaret Joan Misch, Department of Plant Pathology, University of Illinois, Urbana.

Temperature Effects on a Strain of *Rhodotorula glutinis*. P. Thomas Vernier, Wheaton College, Wheaton.

The Isolation of a Mutant of *Bacillus macerans* Lacking Cyclodextrinase. Kandy D. Baumgardner and John A. DePinto, Bradley University, Peoria.

Properties of a Tobacco Mosaic Virus Isolated from Cattleya Orchids. Keramat Izadpanah, Perla H. Canares, Mary Ruth Thompson and H. H. Thornberry, Department of Plant Pathology, University of Illinois, Urbana.

Corn Red Kernel Streak Disease: Causality. H. H. Thornberry and Mary Ruth Thompson, Department of Plant Pathology, University of Illinois, Urbana.

The Influence of Temperature on the Pathways of Glucose Catabolism in *Pseudomonas fluorescens*. S. A. Palumbo and Lloyd D. Witter, Department of Food Science, University of Illinois, Urbana.

Flora and Fauna Present in the North Branch of the Chicago River, 1967. Carolyn Nelson, R. Pearson, N. Hagberg and E. J. Kennedy, North Park College, Chicago.

PHYSICS

Room 0226, Science Building
William C. Shaw, Chairman
Southern Illinois University, Edwardsville

Lab-less Experiments. William C. Shaw, Southern Illinois University, Edwardsville.

The Illinois State Physics Project, an N.S.F. Funded Program of Improvement. Ralph J. Miller, Greenville College, Greenville.

The Use of Segmented Intermolecular Models. John Kenny, Bradley University, Peoria.

A Study of Binary Intermetallic Compounds. Lionel K. Walford and Richard L. Kerch, Southern Illinois University, Edwardsville.

The Compton Profile of Berylium. Lionel K. Walford and Barbara G. Brooks, Southern Illinois University, Edwardsville.

A Simple Model for Certain Electron Beam Devices, with an Application to Saturation in Traveling Wave Amplifiers. Herbert H. Snyder, Southern Illinois University, Carbondale.

Kinetic Energy Described as a Vector. Leo Seren, Aurora College, Aurora.

Searching for the Magnetic Monopole. Otis B. Young, Southern Illinois University, Carbondale.

Annihilation Phenomena in Classical Physics. Leo Seren, Aurora College, Aurora.

Angular Dependence of 2^3S Excitation of He by High Energy Electron Impact. Ik-Ju Kang and Chan Kyoo Choi, Southern Illinois University, Carbondale.

Acoustic Birefringence in Liquids. W. R. Klein and W. A. Riley, Southern Illinois University, Carbondale.

SCIENCE TEACHING

Room 1212, Science Building
Howard T. Davis, Chairman
Normal Community High School, Normal

KEYNOTE ADDRESS: Does Science Curriculum Revision End Here? John R. Carlock, Illinois State University, Normal.

A Novel Approach in Teaching First Concepts of Science: Henry Harrison Owl, the Sad Water Molecule. Edward M. Fashing, University of Illinois, Chicago Circle.

The Ubiquitous Plastic Bottle. John R. Carlock and Harold A. Moore, Illinois State University, Normal.

The Take Off Rearrangement of Chemical Curricula Adjusted for Classes Which Have Enrolled in Chemistry, Biology, Mathematics and Medical Technology. Sister M. Joan Preising, College of Saint Francis, Joliet.

Experiments in the Use of Slides in Teaching of Molecular Structure. Gordon C. Bliss, Southern Illinois University, Edwardsville.

ZOOLOGY

Room 1210, Science Building
Florence M. Foote, Chairman
Southern Illinois University, Carbondale

Electron Microscope Observations of the Aortic Arch of the Earthworm, *Lumbricus terrestris*. Norton B. Gilula and Harold M. Kaplan, Southern Illinois University, Carbondale.

Carbon Studies Done on Cells Found in Hemolymph of the Slug, *Limax maximus*. Shirley Moore, Illinois State University, Normal.

Pituitary Sialic Acid During the Estrous Cycle of the Rat. D. Reed Jensen, Illinois State University, Normal.

Correlation of Defective Maze Learning and Abnormal Electroencephalograms in Phenylketonuriac Mice. L. A. Nadalo, D. C. Tungseth, and E. J. Kennedy, North Park College, Chicago.

Minimum Energy Demands and Metabolic Rates of Short-tailed Shrews, *Blarina brevicauda*. David L. Martinsen, Elmhurst College, Elmhurst.

Forelimb Determination in the Salamander, *Ambystoma tigrinum*. Nancy R. Parker, Southern Illinois University, Edwardsville.

The Effects of Thalidomide on the Development in vitro of the Knee Joint of the Japanese Quail (*Coturnix coturnix japonica*). Allen W. Jacobs, and Florence M. Foote, University of Iowa, Iowa City, and Southern Illinois University, Carbondale.

Determination of Nervous Control and Activity of Myoepithelial Cells of Rat Salivary Glands. Jim N. Tone, Illinois State University, Normal.

Factors Affecting Spontaneous Ejaculation of Male Guinea Pigs. Jan Martan, Southern Illinois University, Carbondale.

Concentrations of Plasma Constituents at Various Body Temperatures in the Lizard, *Dipsosaurus dorsalis*. Mary Lou Haun and Edgar C. Gasdorf, Bradley University, Peoria.

Variations in Vocalizations Produced by the Giant South American Toad, *Bufo blombergi*. Lauren E. Brown, Illinois State University, Normal.

Certain Metabolic Factors Involved in Seminal Fructose Formation. Edward P. Wallen and E. A. Hess, Northern Illinois University, DeKalb.

A Comparative Study of Uterine Motility in the Rat. Joseph D. Bast, and Edwin A. Hess, Northern Illinois University, DeKalb.

Aspects of Oxytocin Release Related to Uterine Contractility in the Rat. Craig A. Miller and E. A. Hess, Northern Illinois University, DeKalb.

Section B

Room 1224, Science Building
Justin N. Frost, Presiding
Southern Illinois University,
Edwardsville

Food Caching by Golden-Mantled Ground Squirrels, *Citellus lateralis*. Bert

A. Landes, Eastern Illinois University, Charleston.

Concerning Type Localities and Collection Sites. Hugh C. Rawls, Eastern Illinois University, Charleston.

Collembola Found on Mississippi River Flood Plain. Fr. John L. Ostdiek, O.F.M., Quincy College, Quincy.

The Communicative Function of Signal Movements in the Parent-Young Interactions of the Cichlid Fish, *Etroplus maculatus*. James E. Cole and Jack A. Ward, Illinois State University, Normal.

Gene Action in *Drosophila melanogaster*: Chromatographic Comparison of Pteridines in the White Locus Alleles w, w^h, w^{co}, w^e, w^{ap}, and w^{ap^2}. Katherine Wilson Bennett and Jack Bennett, Northern Illinois University, DeKalb.

Competition of White Locus Alleles of *Drosophila melanogaster*: w and w^a. Ron Ostrowski and Jack Bennett, Northern Illinois University, DeKalb.

Behavioral Genetics of *Drosophila melanogaster*: Response of White Locus Alleles to Color, Temperature, Direction, and Magnetic Field Differences. Gregory B. Stanton and Jack Bennett, Northern Illinois University, DeKalb.

Nutritional Studies in *Drosophila*: Culture Media for *D. tripunctata*. James P. Weeks and Jack Bennett, Northern Illinois University, DeKalb.

Serological Studies with the Domestic Chicken: Fluorescent Labelled Antibodies and Localization of Tissue Antigens. Leslie D. Waters and Jack Bennett, Northern Illinois University, DeKalb.

Distribution of Helminth Parasites in Turtles Native to Southern Illinois. Dorothy R. Marbin, Monmouth College, Monmouth.

Some aspects of the Effects of Hyperthermia on the Integrity of Cellular Organelles in *Tetrahymena*. Michael R. Levy, Southern Illinois University, Edwardsville.

Intracellular Localizations of Acid Phosphates in *Tetrahymena*. Alan L. Loeh, Southern Illinois University, Edwardsville.

Respiratory Effect of Depressant Drugs on the Laboratory Mouse. Lester C. Shell, G. Dixon, M. Wayne, C. Schaeffer, and T. Kraemer, Millikin University, Decatur.

THE ANNUAL BUSINESS MEETING

The Business Meeting was called to order at 5:00 P.M., April 26, 1968, in the Science Auditorium, Southern Illinois University, Edwardsville, by President Milton Thompson. Approximately 60 members were present.

Reports of Officers. — Secretary Liberta distributed copies of the Librarian's report and the Budget Committee's report to the members. A motion was made and passed to accept these reports.

The Treasurer, Boris Musulin, distributed copies of his report for fiscal 1967, published herewith, and a motion was made and passed to accept his report.

Reports of Standing Committees. — David G. Rands, Chairman of the Research Grants Committee, recommended that the following grants be awarded:

Mr. Ronald L. Koteskey — $200.00. Greenville College. Equipment and supplies for a study of behavioral factors regulating population growth in deer mice.

Mr. Roderick R. Irwin — $75.00. 24 E. 9th Place, Chicago, Illinois. Travel.

Dr. Benedict J. Jaskoski — $100.00. Loyola University. Supplies for the study of the chemistry of egg shells of the tick, *Rhipicephalus sanguineus*.

Dr. Carl Weatherbee — $150.00. Millikin University. Materials and services for analytical determinations.

Dr. Emanuel Merdinger — $200.00. Roosevelt University. Chemicals for the study of transformation of steroids by *Pullularia pullulans* and *Debaryomyces hansenii*.

Dr. D. J. McWhinnie — $150.00. DePaul University. Animals for use in a study of regulation of bone development by pituitary hormones in the chick embryo.

Mr. Gilbert K. Boese — $150.00. Elmhurst College. Tape recorder and accessories for recording the effects of space deprivation on the male hierarchy in captive baboons.

Sr. Mary Marina Kennelly — $150.00. Mundelein College. Chemicals for structural studies of metal complexes of amino acids.

Cynthia Wright — $75.00. University of Illinois. Travel for a paleoecological investigation of the Oak Grove beds, Middle Pennsylvanian, in western Illinois.

Dr. Frank B. Kulfinski — $100.00. Illinois Wesleyan University. Purchase of a planimeter to use in the study of changes in nuclear, nucleolar, and cell size with senescence, development, and disease.

A motion was made and passed to approve the grants. Mr. Rands also stated

that his committee would begin an investigation into the possibilities of instituting a centralized source of information about surplus research equipment that would be available at various colleges and universities, as well as exploring the possibility of the co-operative use of expensive items of research equipment.

Reports of Special Committees.—G. R. Yohe, Chairman of the Permanent Central Office Committee, reported that his committee had been preparing a grant proposal during the past year, and that it was nearly ready for use in approaching various philanthropic organizations for funds to support the Central Office.

Walter B. Welch, Chairman of the Nominations Committee, presented his committee's slate of officers and committee chairmen to the members. All nominees were elected by a unanimous vote. A list of the officers, standing committee personnel, and section chairmen for 1968-1969, is included herewith.

The Chairman of the Planning Committee, Miss Joan Hunter, introduced the proposed constitutional amendments for a vote by the members present. All of the proposed amendments were passed, and these amendments are listed below.

Article III, Section 1 was revised as follows: the second sentence was revised to read "Any organization or person approved by the Chairman of the Contributing Membership Committee may become a contributing member upon payment of the appropriate dues."

Article III, Section 4 was revised as follows: the categories of sustaining and patron member were deleted, and "Contributing member, annual dues $10.00 or more," was substituted. The category of "Emeritus member, no dues," was added.

Article III, Section 5 was revised as follows: the second sentence was rewritten to read "Proceeds from the contributing memberships, over and above the amounts paid by regular members, shall be used to finance special activities of the Academy and to advance its program as shall be determined by the Council."

Article III, new Section 6: "An emeritus member shall be one who has been a member of the Academy for at least ten years and is retired (from his regular occupation)."

Article III, new Section 7: Old Section 6 was revised to read "The fiscal year of the Academy shall be from January 1 through December 31. Members who fail to pay dues for any fiscal year shall be considered in arrears for that year."

Article V, Section 1 was revised as follows: in the first sentence, "four" Councilors-at-large was changed to "twelve." The second sentence was changed to read "these last shall be elected to three year terms, four being elected each year until there are twelve."

Article II, Section 1 of the By-laws was changed as follows: "Legislation and Finance" became "Finance," and "Sustaining Memberships" became "Contributing Memberships."

Harold M. Kaplan, Chairman of the Resolutions Committee, submitted the following resolutions; all were approved by the members of the Academy:

1. APPRECIATION TO THE HOST — *Whereas* many members of the Administration, Faculty, and Staff of Southern Illinois University have been responsible for the arrangements essential to the Sixty-first Annual Meeting of the Illinois State Academy of Science, *Be it resolved* that the Academy express its gratitude to all who have participated in any way in arranging this Annual Meeting, and especially to:
   Dr. Delyte W. Morris, President of the University, and;
   Dr. William J. Probst, Second Vice President of the Academy, in charge of local arrangements.
   Be it further resolved that the Secretary of the Academy be directed to send copies of this resolution to those specifically named.

2. APPRECIATION OF SERVICE — *Whereas* responsibility has been accepted by Drs. Paul D. Kilburn and John Wanamaker of the Department of Biology, Principia College, for arranging the Botany Field Trip, and *Whereas* Dr. Emily Blasingham, Curatory of Anthropology at the Illinois State Museum, Dr. Robert Hall of the Department of Sociology and Anthropology at Marquette University, and Dr. Charles J. Bareis, Anthropologist, University of Illinois, are cooperating in leading an Archæological Field Trip to Cahokia, and *Whereas* these Field Trips constitute a significant portion of the program of the Sixty-first Annual Meeting of the Academy, *Be it resolved* that the Academy express its thanks to these individuals for organizing and conducting the

Field Trips, and *Be it further resolved* that the Secretary of the Academy be directed to send a copy of this resolution to those specifically named.

3. APPRECIATION OF SERVICE — *Whereas* Dr. Anthony E. Liberta, of the Illinois State University, has served the Academy in a most worthy and highly productive manner for three years as Secretary of the Illinois State Academy of Science, *Be it resolved* that the members of the Academy express their appreciation for his outstanding service, and *Be it further resolved* that the Academy President be directed to send him a letter of commendation, and *Be it further resolved* that copies of this Resolution and the letter of commendation be sent to President Samuel Braden, Dean of the Faculty Richard R. Bond, and Dean of the College of Liberal Arts and Sciences, James K. Olsen, all of the Illinois State University.

4. CONSERVATION OF NATURAL RESOURCES — *Whereas* the total destruction of our remaining natural areas and their resource values for our own and future generations are threatened with the pressures of population, and *Whereas* the preservation of our natural heritages is essential for the pursuit of scholarly study and the welfare of the people of the State, *Be it resolved* that the Academy commend the kind of study which has recently been carried out by the Technical Advisory Committee on Water Resources for the State of Illinois, and *Be it further resolved* that the Academy recommend broad, multidisciplinary studies of all the natural resources of the State, and *Be it further resolved* that the Academy state the opposition of its members to impoundment or development plans by any governmental agency which have not been proposed with due consideration to the values of conservation and natural resources for the perpetual benefit and enjoyment by the people of this State, and *Be it further resolved* that the Secretary of the Academy send copies of this Resolution to Governor Otto Kerner, and to Dr. William C. Ackermann, Water Plan Director, for distribution to his Committee.

5. CONSERVATION OF THE NATURAL RESOURCES OF LUSK CREEK CANYON—*Whereas* Lusk Creek Canyon in the Shawnee Hills of southern Illinois is a priceless, irreplaceable natural resource for botanical, zoological, geological, and archæological values and study, and *Whereas* this Canyon has been recommended for a Natural Area status by the Illinois Technical Advisory Committee on Water Resources in its 1967 report, "Water for Illinois, A Plan for Action", and *Whereas* the Forest Service of the U. S. Department of Agriculture has proposed an impoundment which would destroy or greatly impair the above values of the Canyon, and *Whereas* we as citizens and scientists have a paramount obligation to present and future generations to conserve our resources for the best possible uses, *Be it resolved* that the Academy request the elected officials of the people of Illinois and the heads of the Illinois Department of Conservation, Illinois State Geological Survey, Illinois Natural History Survey, Illinois State Water Survey, and the Illinois Nature Preserves Commission to assist in creative, long-range planning for the Lusk Creek drainage basin and to request of the Forest Service further consideration of the proposed impoundment, and *Be it further resolved* that the Secretary of the Academy send copies of this Resolution to Governor Otto Kerner, Senator Everett M. Dirksen, Senator Charles H. Percy, Representative Kenneth J. Gray, Director William T. Lodge, Dr. John C. Frye, Dr. George Sprugel, Jr., Dr. William C. Ackermann, and Dr. Willard D. Klimstra.

6. PRESERVATION OF SCIENTIFIC STUDY AREAS NEIGHBORING THE OAKLEY RESERVOIR—*Whereas* the Illinois State Academy of Science is deeply concerned with the destruction of important scientific study areas in Allerton Park that would result from the construction of the Oakley reservoir, therefore *Be it resolved* that the Illinois State Academy of Science support the request originated by the Illinois Nature Preserves Commission which would delay the spending of any monies appropriated for land acquisition and construction of the reservoir until a

detailed restudy is made of alternative plans to prevent this destruction by returning the pool level to the original 621 feet, and *Be it further resolved* that the Secretary of the Illinois State Academy of Science be directed to send a copy of this Resolution to Governor Otto Kerner, to the Secretary of Defense, to the Secretary of the Interior, to members of the Congress of the United States, to the President of the University of Illinois, to the Corps of Engineers, to the State Water Survey, and to the State Sanitary Water Board.

7. NECROLOGY — *Whereas* during the past year the Academy has lost by death: Donald T. Ries, Richard R. Kudo, William S. Shonka, C. M. Goethe, Louis A. Astell, and Garret W. Thiessen, *Be it resolved* that the Academy give expression of its sorrow for the deceased members by rising for a moment of silence.

Anthony E. Liberta
Secretary

OFFICERS AND STANDING COMMITTEE NOMINEES FOR 1968-1969

President: William C. Ashby, Southern Illinois University, Carbondale.
First Vice President: Harold M. Kaplan, Southern Illinois University, Carbondale.
Second Vice President: Carl Weatherbee, Millikin University, Decatur.
Secretary: Kenneth Harmet, Northern Illinois University, DeKalb.
Treasurer: Boris Musulin, Southern Illinois University, Carbondale.
Librarian: Milton D. Thompson, Illinois State Museum, Springfield.
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William J. Probst (to 1971), Southern Illinois University, Edwardsville.
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TREASURER'S REPORT — JAN. 1 - DEC. 31, 1967

Receipts and Disbursements

Receipts Jan. 1 — Dec. 31, 1967				$ 6,109.92
Dues			$ 3,947.00	
Regular	1966	$ 20.00		
	1967	1,676.00		
	1968	1,975.00		
Student	1967	82.00		
	1968	68.00		
Emeritus	1967	7.00		
	1968	19.00		
Sustaining	1967	30.00		
	1968	70.00		
Life		.....		
Library subscriptions			$ 257.53	
AAAS research grant reimbursement			506.00	
Interest—U.S. Bonds & Notes			279.61	
Interest—Savings & Loan Accounts			957.78	
Registration Fees (Annual Meeting)			138.00	
Miscellaneous			.....	
Sale of publications			1.00	
Gain on Investments			23.00	
Disbursements Jan. 1 — Dec. 31, 1967				$ 4,938.89
Council			$ 353.91	
Editor's Office			160.85	
Frank H. Reed Memorial Prize			75.00	
Honoraria			500.00	
Junior Academy of Science			15.00	
Science Talent Search			490.80	
Librarian			369.40	
Membership Committee			305.73	
Miscellaneous Expenses			24.52	
Research Grants			1,350.00	
Secretary's Office			802.84	
Treasurer's Office			490.84	
Net Income for 1967				$ 1,171.03

Checking Account Balance

Checking account balance Jan. 1, 1967		4,303.42
Deduct $200.00 Permanent Fund monies		200.00
Real checking account balance		4,103.42
Net income		1,171.03
Checking account balance Jan. 1, 1968		5,274.45
Less outstanding checks +$23 gained & reinvested		237.45
Official checking account balance Jan. 1, 1968		5,037.00

Statement of Assets Dec. 31, 1967

Official checking account balance		$ 5,037.00
Permanent Fund		2,350.00
U.S. Government Bonds	$ 2,000	
Carbondale Savings & Loan Acct. #3522	350	
Reserve Funds		19,500.00
U.S. Bonds & Treasury Notes	4,500	
Carbondale Savings & Loan Acct. #3522	6,000	
State Savings & Loan Acct. #435	9,000	
Frank H. Reed Memorial Fund		1,455.00
TOTAL ASSETS		$28,342.00

Boris Musulin
Treasurer

New York Botanical Garden Library
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PREPARATION OF MANUSCRIPTS FOR THE TRANSACTIONS

For publication in the *Transactions*, articles must present significant material that has not been published elsewhere. Review articles are excepted from this provision, as are brief quotations necessary to consider new material or varying concepts. All manuscripts must be typewritten, double spaced, with at least one-inch margins. The original copy and one carbon copy should be submitted.

Titles should be brief and informative. The address or institutional connection of the author appears just below the author's name. An abstract must accompany each article. Subtitles or center headings should be used; ordinarily one uses subtitles such as *Materials*, *Methods*, *Results*, *Discussion*, *Summary*, *Acknowledgments*, and *Literature Cited*.

No footnotes are to be used except in tables.

The section entitled *Literature Cited* must include all references mentioned in text. It is not to include any other titles. Citations under *Literature Cited* are as shown below:

Doe, J. H. 1951. The life cycle of a land snail. Conchol. 26(3): 21-32, 2 tables, 3 figs.

Doe, J. H., and S. H. Jones. 1951. Mineralogy of Lower Tertiary deposits. McGraw-Hill Book Co., New York. iv + 396 pp.

Quoted passages, titles, and citations must be checked and rechecked for accuracy. Citations to particular pages in text are Doe (1908, p. 21) or (Doe, 1908, p. 21); general citation in text is Doe (1908) or (Doe, 1908).

Tabular information should be kept at a minimum. Do not duplicate tabular data in text. Headings for tables and columns should be brief. Each table and its heading should be on a single page; do not place any table on the same page with text.

Photographs should be hard, glossy prints of good contrast. Graphs, maps and other figures reproduce best when prepared for at least one-half reduction; lettering, numerals, etc. on all figures in a manuscript should be worked out to proper size for such reduction. Line widths, letter size, etc., should be uniform from figure to figure within a published paper. Figures should be drawn on good quality white paper or on drawing board. Use only India ink. Use a lettering device (Leroy or Wrico) for numerals and words; do not print "free-hand."

Legends for photographs and figures should be brief; type them on a separate sheet of paper. Indicate figure number and your name on back of illustration; do not write with pencil on the backs of photographs.

Authors will receive galley proofs; these should be read carefully and checked against the original manuscript. Reprints may be ordered at the time galley proofs and manuscripts are returned to the Editor.

Darrel L. Lynch,
Department of Biological Sciences,
Northern Illinois University,
DeKalb, Illinois 60115

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